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JOURNAL

OF THE

TOKYO UNIVERSITY OF FISHERIES

VOL. 45, NO. 2.

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THE TOKYO UNIVERSITY OF FISHERIES
SHIBA KAIGANDŌRI 6, MINATO-KU, TOKYO, JAPAN
March, 1959.

JOURNAL OF THE TOKYO UNIVERSITY OF FISHERIES

Editors for this Journal

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Journal of the Tokyo University of Fisheries, Vol. 45, No. 2, 1959

POLAROGRAPHIC STUDIES OF PROTEIN CONTAINED IN AQUATIC ANIMAL

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(Received Feb. 14, 1959)

Introduction

Many of the papers have been published on the subject of polarographic study of protein, but the polarographic characteristics of proteins contained in fish muscle are not known so far. Therefore, in the present paper, the authors tried the application of polarographic protein wave to the studies of proteins contained in fish muscle. Here the following subjects were studied.

1. Preliminary experiment.
2. Detection of typical protein wave.
3. Origin of the catalytic wave produced in water extractives of meat.
   1) Catalytic wave of meat extractives before and after heating.
   2) Catalytic wave of alcoholic extractives of meat.
   3) Separation of original substance by alcoholic treatment.
4. Specific difference of aquatic animal shown in protein wave.
   1) Comparative observation of several aquatic animals.
   2) Comparative observation of reddy meat and white meat.
   3) Polarographic characteristics of meat for several aquatic animals.
5. Comparative studies of common muscle and dark colored muscle by polarographic protein wave.
   1) Comparative observation of several common muscle and dark colored muscle.
   2) Interference of meat extractives for albumin-protein wave.
   3) Substance to interfere the protein wave.
6. A trial of the determination for meat freshness.
7. Denaturation of protein by γ-ray irradiation.
   1) Influence of γ-ray irradiation to serum.
   2) Influence of γ-ray irradiation to albumin.
   3) Influence of γ-ray irradiation to globulin.
   4) Influence of γ-ray irradiation to fish meat.

Since the discovery of catalytic wave by HERLES[1], HEYROVSKÝ and BRDIČKA[2], the application of catalytic wave have been developed in many field. In 1933, BRDIČKA[3] found double protein wave in serum having the potentials of -1.4V and -1.8V in ammoniacal cobaltic solution.

The protein wave clearly present in mixed buffer solution containing cobalt salt, NH_4Cl, NH_4OH. Proteins which is presented the protein wave are serum albumin, globulin, egg white, insuline, winevinegar, natural wine and water soluble protein contained in wool. But gelatine, fermented vinegar and water solube protein containe in pure silk does not produce the wave[4]. That is, the proteins which is contained cystine in the component of amino acid produce the

protein wave, and the other protein which do not contained cystine and cystine in the component do not show the protein wave.

BRDIČKA[5] found a catalytic wave of cystine and cysteine in ammoniacal buffer solution. The differences of cystine wave and protein wave are as follows:

1) Protein wave is double wave and cystine wave is single wave. The potential of the latter is almost the same as the potential of the second wave of the former.

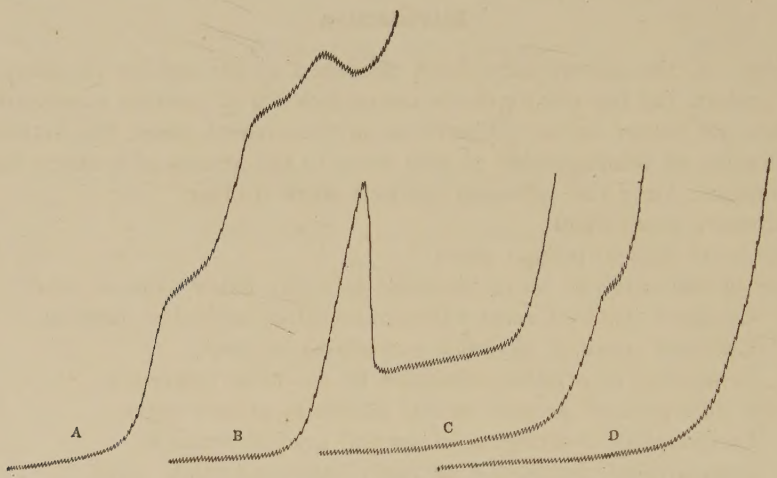


Fig. 1. Polarographic catalytic waves.
A. protein wave, 1×10^{-3} M $CoCl_2$+0.1 M NH_3+0.1 M NH_4Cl+dil. serum.
B. 1×10^{-3} M $CoCl_2$+0.1 M NH_3+0.1 M NH_4Cl.
C. prenatrium wave, 0.1 M NH_3+0.1 M NH_4Cl+dil. serum.
D. 0.1 M NH_3+0.1 M NH_4Cl.

2) Protein wave appear in ammoniacal cobaltic solution containing divalent and trivalent cobalt, but cystine wave appear only in ammoniacal cobaltic solution containing divalent cobalt.

The mechanical originations of the catalytic wave are not completely understood, though BRDIČKA[6,7] suggested the original of the catalytic wave due to SH group.

The catalytic waves are observed for cystine, cysteine, homocystine, homocysteine[8], vitamin B_1[9], penicillamine ($\beta\beta$-dimethyl cysteine)[10], mastard gass ($\beta\beta$-dichlorosulfide)[11], glutathione[12], cysteylglycine and thioglycolic acid containing sulfhydryl or disulfide group. The others such as glycine, asparatic acid, creatine, creatinine, arginine, leucine and tyrosine do not show catalytic wave[13]. β-pheny β-alanine[14] produce a catalytic wave though it has not contain sulfer in the molecule.

Such catalytic protein wave is used for applied purpose, namely one is the serological appication of the catalytic waves for diagnosis of cancer[15].

Studies, according to the denaturation of proteins for the irradiation of ultraviolet ray[16-18], X-ray[19], urea[20-23], were reported by many investigators. Other analytical application of the catalytic wave to study the content of cystine in casein[24], characteristics of cow milk[25,26], albumin, globulin and other protein in

vegetable[27,28] extractives of internal organs[29], insuline[30,31], cerbrospinal fluid[32-34], saliva[35] and lachrymal fluid[36] were already reported.

1. Preliminary experiment

This chapter explains the preliminary experiment to examine the protein contained in aquatic animals by polarographic protein wave.

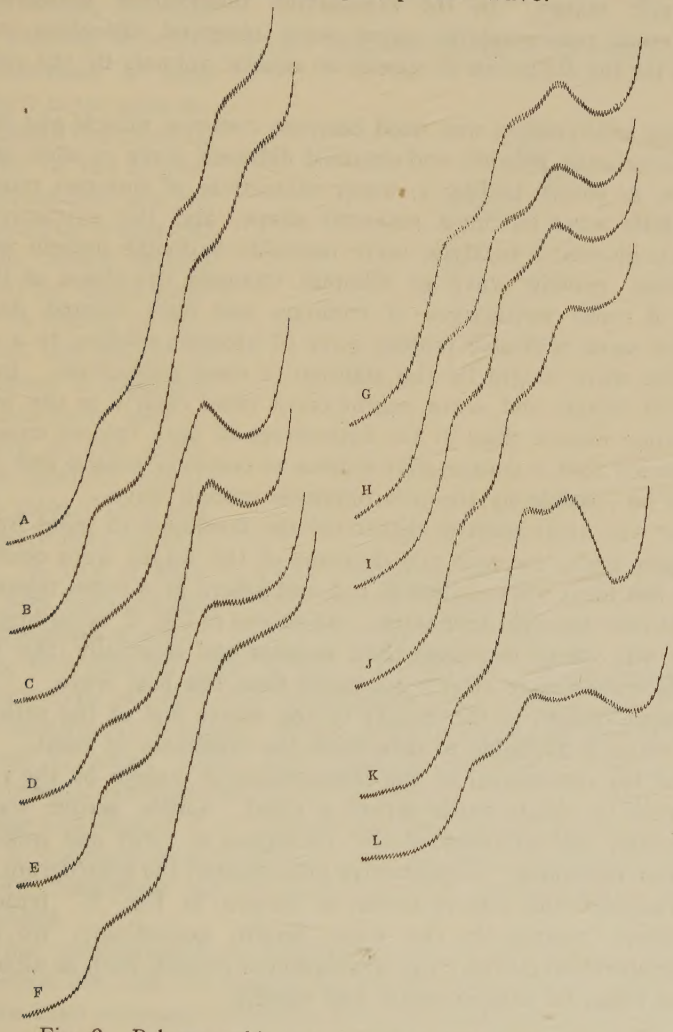


Fig. 2. Polarographic protein waves (representative).
A, C, difference in the species of aquatic animal. A, extractives of *Meretrix meretrix lusoria*; C, extractives of *Katsuwonus pelamis*.
B–F, comparison of common muscle and dark colored muscle of *Katsuwonus pelamis*. B, 0.2% albumin soln.; C, extractives of common muscle; D, extractives of common muscle+0.2% albumin soln.; E, extractives of dark colored muscle; F, extractives of dark colored muscle+0.2% albumin soln.
G–J, change of the protein wave height with the freshness, G, 0; H, 1; I, 3; J, 5 days elapsed at 7–12°C. The water extractives of cuttle fish muscle was used for this experiment, and applied the filtrate reaction technique.
K, L, effect of γ-ray radiation to the dil. serum, K, 3×10^5 r; L, control.

1) For the study of specific difference, muscle of *Katswonus pelamis* and *Meretrix meretrix lusoria* were prepared. Both muscle were extracted with water and polarographed by the addition of ammoniacal cobaltic solution involving divalent cobalt. As shown in Fig. 2, water extractives of *Katswonus pelamis* showed a single catalytic wave and the extractives of *Meretix meretrix* showed double catalytic waves. In the compartive observation between fish and mollusca different polarographic waves were observed, therefore we expected further study for the difference of species of aquatic animals by the polarographic wave.

2) Same examination was tried between common muscle and dark colored muscle of *Katsuwonus pelamis*, and obtained different wave in each other.

That is, as shown in Fig. 2, water extractives of common muscle showed a single catalytic wave having a maximal shape, and the extractives of dark colored muscle showed a catalytic wave resemble to double protein wave.

Otherwise, protein wave of albumin changed the shape of the wave by the addition of water extractives of common and dark colored muscle. The change of the wave is double protein wave of albumin solution to a single wave and reduce the wave height by the addition of meat extractives. In this case, the change of shape and wave height occur more clearly in the water extractives of common muscle than in the extractives of dark colored muscle. Therefore we expected that a comparative studies of common muscle and dark colored muscle could be possible by the polarographic protein wave.

3) For the application to determine the freshness of meat by the polarographic protein wave, increase and decrease of the waves were observed.

Cuttle fish meat were elapsed 0, 1, 3 and 5 days at a room temperature, and tested by filtrate reaction technique. As shown in Fig. 2, according to decrease of freshness, the waves increased their heights and especially the second wave of the double waves more clearly increased than the first wave.

The measurement of the height of the waves and of the ratio of the first and second wave is available to determine the freshness of meat.

4) For the description of the denaturation of protein by the γ-ray, irradiation experiment for dilute cuttle serum is cited. Cuttle serum was diluted 50 times with water and irradiated 3×10^5 roentgens of γ-ray and polarographed by filtrate reaction technique. Comparative difference of the polarogram was obtained between irradiated and control serum as shown in Fig. 2. Irradiated serum showed a clearly change in the wave height, accordingly, we expected the studies of denaturation by the γ-ray irradiation of protein such as albumin, globulin and fishmeat could be studied easily and rapidly.

2. Detection of typical protein wave

For the studies of protein involved in the muscle of aquatic animals by polarographic catalytic wave, it is necessary to get a typical catalytic wave. This chapter explains the result of the experiment object to obtain a typical catalytic wave for the water extractives of aquatic animal meat.

Apparatus: The used polarograph is selfrecording type manufactured by Yanagimoto Co. having the highest sensitivity of 0.002 μA/mm.

Basic solution: Three kinds of common basic solutions are used, and simplify to description these are called as follows:

Basic solution	Component
ammoniacal cobaltic (II) soln.	1×10^{-3} M $CoCl_2$, 0.1 M NH_4Cl, 0.1 M NH_4OH
ammoniacal cobaltic (III) slon.	1×10^{-3} M $[Co(NH_3)_6Cl_3]$, 0.1 M NH_4Cl, 0.1 M NH_4OH
ammoniacal cobaltic (III) soln. for filtrate reaction technique	1×10^{-3} M $[Co(NH_3)_6Cl_3]$, 0.1 M NH_4Cl, 0.8 M NH_4OH

Measurement of wave height: As it is not quite definite to measure the catalytic wave height, in the present paper it makes into one the height of catalytic waves, HI and HII for double protein waves and hI for single catalytic wave as shown in Fig. 3. That is, the height of catalytic waves is measured from the maximum part of cobaltic wave to the maximum of the catalytic wave.

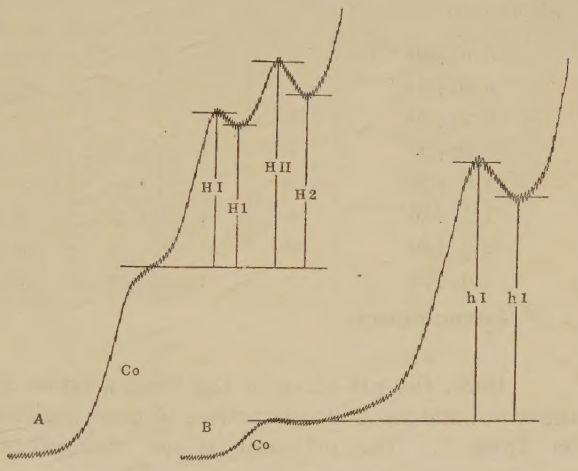


Fig. 3. Method for the determination of waveheight of protein wave and cystine wave.
A, protein wave; B, cystine wave

Fresh meat of carp and cuttle fish were used for this experiment. A 10 g of each fresh meat was homoginized with small quantities of water and fulled up with distilled water to 100, 250, 500 and 1000 ml. respectively, then filtered. Electrolytic solution was prepared by the addition of the filtrate and ammoniacal cobaltic (II) solution. Polarograms of four kinds of water extractives for two kinds of meat, added with different quantities of filtrate to 20 ml. of ammoniacal cobaltic solution are shown in Table 1.

In Table 1, a sign of " + " means the wave height have more than 20 mm. n height; " ± ", samely about 10 mm.; " − ", less than 10 mm. respectively, max., means cobaltic maximum and in this case any polarographic catalytic wave was not obtained.

In the low concentration of protein produced a maximum of cobalt and with he concentration are rose the maximum are then deminished, and the height of atalytic wave then enlarged according with increasing concentration of protein.

The preparation which is obtained the catalytic wave as shown a sign of + " in Table 1, is suited to get a typical catalytic wave for meat extractives.

For other liquid samples such as serum[39] and drip of frozen whale meat, t is an appropriate method to get a typical wave that they are diluted 50 times vith water and added with 10 times quantities of ammoniacal cobaltic solution.

It is necessary to determine the pH value of the basic solution after the ddition of water extractives of meat, because the catalytic wave is affected by he pH value of basic solution.

Table 1. Height of protein wave of meat extractives.
−, maximum of cobalt; +, > 20 mm.; ±, ca 10 mm.; E., extractives; buffer, ammoniacal cobaltic buffer soln.

Species / Extraction ratio* / E(ml) +buffer(ml)	Carp				Cuttle fish			
	10	25	50	100	10	25	50	100
0.10+20	−	−	−	−	−	−	−	−
0.20+20	−	−	−	−	−	−	−	−
0.25+20	±	±	−	−	±	±	−	−
0.50+20	+	+	−	−	+	+	−	−
1.0 +20	+	+	−	−	+	+	−	−
1.5 +20	+	+	±	±	+	+	±	±
2.0 +20	+	+	+	+	+	+	+	+
3.0 +20	+	+	+	+	+	+	+	+

* extractant/meat.

Here, the pH value of the basic solution added with different quantities of the water and alcoholic extractives of meat were examined. The results are shown on Table 2. The pH value is not changed at least the addition of 4.0 ml. of

Table 2. pH of ammoniacal cobaltic buffer solution involving meat extractives.
E., extractives of meat; buffer, ammoniacal cobaltic buffer soln.; A, water extractives; B, 80% alcohol extractives.

E (ml)+buffer (ml)	A	B
0 +20	9.50	9.50
0.10+20	9.50	9.58
0.25+20	9.50	9.55
0.50+20	9.50	9.55
1.0 +20	9.50	9.52
2.0 +20	9.50	9.50
3.0 +20	9.50	9.45
4.0 +20	9.50	9.42
5.0 +20	9.48	9.40

water extractives and 2.0 ml. of alcoholic extractives for 20 ml. of ammoniacal cobaltic solution respectively.

3. Origin of catalytic wave produced in water extractives of meat

In water extractives of meat, proteins and other substance are involved, these are amino acid, inorganic matter and other low-molecular substance. Above

all the substance which is possible to produced catalytic wave and protein wave, are proteins and a certain low-molecular substance such as cystein, cystine etc. The latter substances do not produce double protein wave in ammoniacal cobaltic (III) solution though they produce a single catalytic wave in ammoniacal cobaltic (II) solution, therefore it is possible to examine the original substance of catalytic wave by a comparative observation of meat extractives in ammoniacal cobaltic (II) and (III) solution.

On the other hand comparative observation of meat extractives before and after heating and separation of proteins by alcoholic treatment is available to examined the original substance in meat extractives.

1) Catalytic wave of meat extractives before and after heating.

Fresh meat extractives of *Limanda yokohamae* are prepared and boiled for 20 minutes and then filtered, the filtrate was tested by the polarographic method. Comparative results for meat extractives before and after heating are shown in Fig. 4.

The filtrate showed a double protein waves though they decrease their waveheight by heating. It means that the original substance is soluble in hot water and also stable to heating. On the other hand the decrease of waveheight indicates that the original substance precipitates in heating.

2) Catalytic wave of alcoholic extractives of meat.

Fresh meat of *Limanda yokohamae* was homoginized and extracted with 10 times quantities of alcohol and then filtered. The filtrate was prepared having the finally concentration of alcohol was exactly 80%. The alcoholic filtrates were polarographed in addition of ammoniacal cobaltic (II) and (III) solution as basic solution. Water extractives of this meat prepared as alcoholic extract were tested too. These results are presented in Fig. 5.

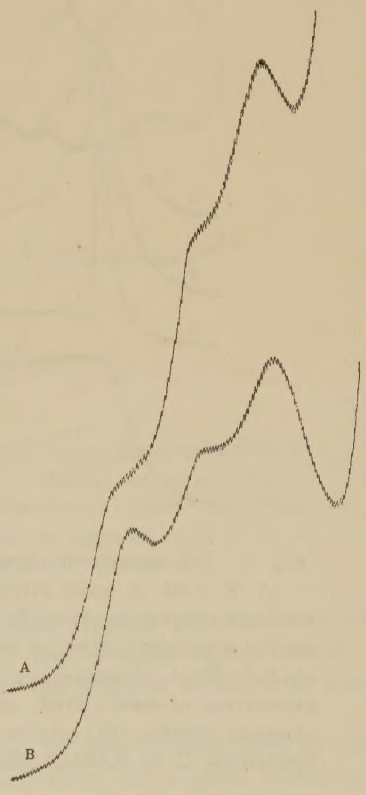


Fig. 4. Protein wave of meat extractives.

A, before heating; B, after heating Ammoniacal cobaltic buffer soln. is involves divalent cobalt. Sensitivity, 0.100 μA/mm.

In all cases alcoholic extractives did not produce any catalytic wave though the water extractives produced cleary protein wave.

In alcoholic extractives of meat low-molecular substance such as amino acid and other sulfer compounds are dissolved, but most of protein can not exist. So the alcoholic extractives produce no catalytic wave, that the low-molecular substance dissolved in the extractives can not to be an original substance of the catalytic wave.

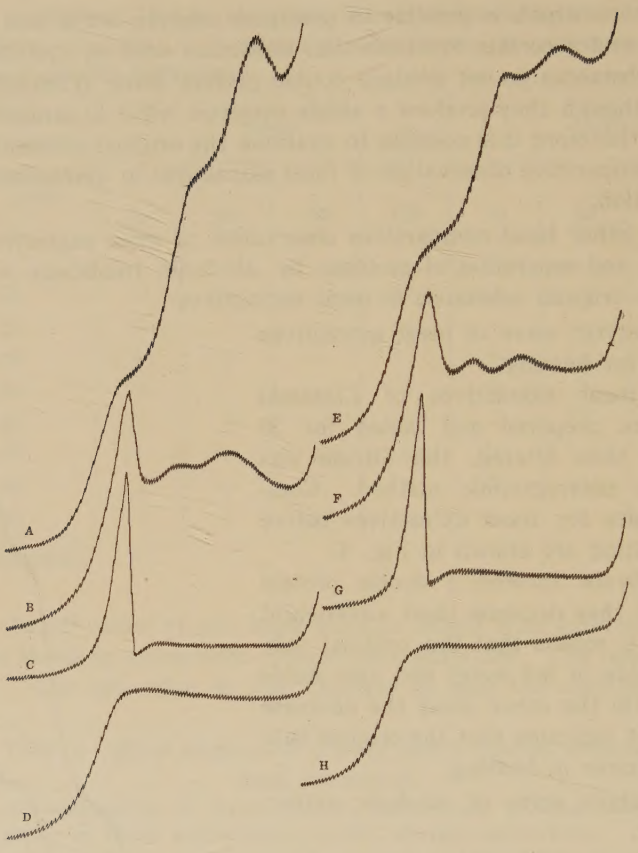


Fig. 5. Polarograms of alcoholic extractives of meat—*Limanda yokohamae*.
A, E, 2 ml. of water extractives of meat (extracted 10 times)+20 ml. of ammoniacal cobaltic solution; B, F, 4 ml. of water extractives of residue by 80% alcohol treatment+20 ml. of ammoniacal cocaltic solution; C, G, 4 ml. of 80% alcohol+20 ml. of ammoniacal cobaltic solution; D, H, 4 ml. of 80% alcoholic extractives of meat+20 ml. of ammoniacal cobaltic solution. A, B, C, D, ammoniacal cobaltic (III) solution; E, F, G, H, ammoniacal cobaltic (II) solution. Sensitivity, C, G, 0.400 μA/mm, others, 0.100 μA/mm.

It is sure that the original substances are contained in the precipitate fraction which is produced by treating the water extractives with 80% alcohol, because alcohol (80%) soluble fraction did not show any catalytic wave and water extractives of the precipitate fraction produce a catalytic wave.

3) Separation of the original substance by alcoholic treatment

In the former experiment the characteristics of the original substance was somewhat clarified. Here the further investigations are done by the separation of the original substance by alcoholic treatment.

Meat extractives (extracted with 10 times of water) of *Penaeus orientalis* and

Thunnus thynnus were prepared and added absolute alcohol. Four kinds of alcoholic solution were made containing finally concentration of alcohol 50, 60, 70 and 80% respectively. Each filtrate in both solution was polarographed in ammoniacal cobaltic (II) or (III) solution. The results are as shown in Figs. 6 and 7.

Fig. 6. Polarograms of alcoholic extractives of meat—*Penaeus orientalis*. A, B, C, D, 2.0 ml. of 50, 60, 70 and 80% alcohol soluble fraction of water extractives of meat (extracted 10 times of water) respectively +20 ml. of ammoniacal cobaltic (II) solution. E, F, G, H, same as A, B, C, D, in ammoniacal cobaltic (III) solution. Sensitivity, 0.100 μA/mm.

The 50, 60 and 70% alcohol soluble fraction produce protein waves, but 80% alcohol soluble fraction does not produce any protein wave, though the maximum of the cobaltic wave is deminished by the addition of the 80% alcohol soluble fraction.

Therefore the substance which dissolved in 80% alcohol such as amino acid and other low-molecular substance exclude from the original substance of catalytic wave. But it is observed that the substance have characteristics to diminish the maximum of cobaltic wave, so it is remained to examine the characteristic of this substance.

It seems that the original substance of catalytic wave in water extractives of meat due to a comparatively low-molecular protein, because most of the high-molecular protein precipitate in the alcoholic concentration of 50 or 60%.

4. Specific difference of aquatic animal shown in protein wave

1) Comparative observation of several aquatic animals.

The components of meat extractives of aquatic animals are different in the

Fig. 7. Protein wave of alcoholic extractives of meat.

A, H, water extractives; B, I, diluted A and H five times; C, J, 50% alcohol soluble fraction of A and H; D, K, 60% alcohol soluble fraction of A and H; E, L, 70% alcohol soluble fraction of A and H; F, M 80% alcohol soluble fraction of A and H; G, N, water extractives of p.p.t. produced by 80% alcohol treatment; A~G, ammoniacal cobaltic soln. involving divalent cobalt; N~H, ammoniacal cobaltic buffer soln. involving trivalent cobalt. Sensitivity, 0.100 μA/mm.

species, therefore it is possible to study the difference of them by polarographic catalytic wave. Here, the comparative observation for several aquatic animals by polarographic catalytic wave is experimented.

It is considerably easy to compare with several kinds of aquatic animals qualitatively, but quantitative comparison is somewhat difficult.

So, in this paper the comparative observation is experimented for several aquatic animals by a limited condition that the additional rate of the 10 times of water extractives of meat and ammoniacal cobaltic (II) solution is 2.0 ml+ 20 ml.

The following 15 species were used for this experiment. Materials of aquatic animals were selected as fresh as possible. In the following description a sign of * means a lived aquatic animals just before the preparation.

I. Fishes

Koi; *Cyprinus caprio* (Linné)*
Katuo; *Katsuwonus pelamis* (Linné)
Saba; *Scomber japonicus* (Houttun)
Maguro; *Thunnus thynnus* (Linné)
Hirame; *Paralichthys Clivaceus* (Temminck et Shlegel)
Karei; *Limanda yokohamae* (Günther)*
Kisu; *Sillago sihama* (Forskål)

II. Mollusca

Surumeika; *Ommatostrephes sloani pacificus* Steenstrup
Yariika; *Doryteus bleekeri* Keferstein
Kaki; *Ostrea* (*Crassostrea*) *gigas* Thunberg*
Hamaguri; *Meretrix meretrix lusoria* (Gmelin)*
Asari; *Venerupis* (*Amygdala*) *philippinarum* (Adams et Reeve)*
Akagai; *Anadara* (*Scaplarca*) *inflata* (Reeve)*

III. Crustaceae

Gazami; *Neptunus* (*Neptunus*) *trituberculates* Miers*
Koraiebi; *Penaeus orientalis* Kishinouge*

A 10 g of each fresh meat was homoginized with small quantities of water and added distilled water to 100 ml, then filtered, the filtrate was used for polarographic electrolytic solution in addition to ammoniacal cobaltic (II) or ammoniacal cobaltic (III) solution.

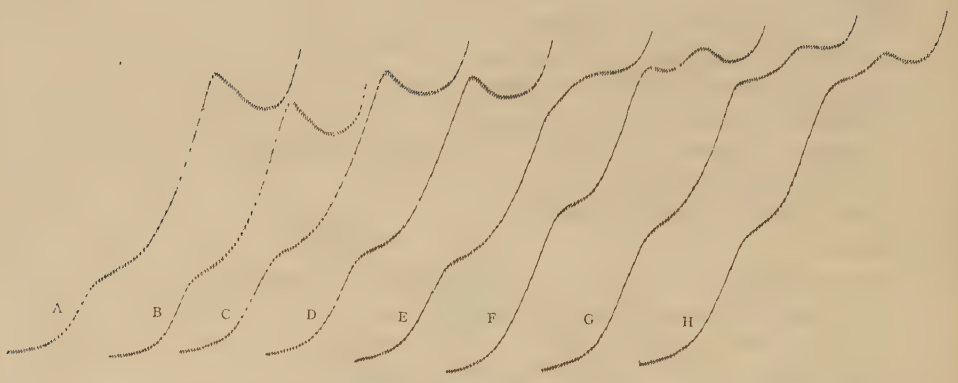
Comparative figures of catalytic waves for meat extractives of aquatic animals are shown in Figs. 8–9.

In the use of ammoniacal cobaltic (II) solution for the basic solution, the protein wave of meat extractives showed a single wave or double protein waves different in the species of aquatic animals. Common fish meat having a reddy color such as *Thunnus thynnus*, *Cololabis saira*, *Katsuwonus pelamis*, etc. showed a single protein wave, and fish meat having a white color such as *Sillago sihama*, *Paralichthys olivaceus* and molluscan and crustacean meat showed double protein wave.

But, in the use of ammoniacal cobaltic (III) solution for the basic solution, all of them produced double protein waves even in fish meat which was produced single protein wave in ammoniacal cobaltic (II) solution. Accordingly it is not possible to show the specific difference of aquatic animals by the protein wave which is produced in ammoniacal cobaltic (III) solution. The height of protein wave of extractives in both basic solution, white meat of fish, mollusca and crustacea are produced higher than reddy meat and the height of protein wave in ammoniacal cobaltic (III) solution is always higher than in ammoniacal cobaltic (II) solution.

Liquid samples such as egg white, cow milk, serum, and drip of frozen whale meat showed double protein wave in both basic solutions.

In this experiment we could not found the specific difference of aquatic animals by the catalytic wave in details, but it was possible to classify the aquatic animals into two groups by the shape of catalytic wave, the one group which was produced single protein wave such as *Thunnus thynnus* and *Cololabis*



A, *Katsuwonus pelamis* (LINNÉ); B, *Scomber japonicus* HOUTTUYN; C, *Cololabis saira* (BREVOORT); D, *Thunnus thynnus* (LINNÉ); E, *Cyprinus caprio* (LINNÉ); F, *Limanda yokohamae* (GÜNTHER); G, *Paralichthys olivaceus* (TEMMINCK et SCHLEGEL); H, *Sillago sihama* (FORSKÅL).

I, *Venerupis* (*Amygdala*) *philippinarum* (ADAMS et REEVE); J, *Meretrix meretrix lusoria* (GMELIN); K, *Ostrea* (*Crassostrea*) *gigas* THUNBERG; L, *Ommatostrephes sloani pacificus* STEENSTRUP; M, *Doryteuthis bleekeri* KEFERSTEIN; N, *Penaeus orientalis* KISHINOUGE; O, *Neptunus* (*Neptunus*) *trituberculatus* MIERS; P, Egg white; Q, Cow milk; R, Blood of carp; S, Drip of frozen whalemeat.

Figs. 8 and 9. Polarographic protein wave of meat extractives.
Used ammoniacal cobaltic soln. contained divalent cobalt. Sensitivity, 0.100 μA/mm.

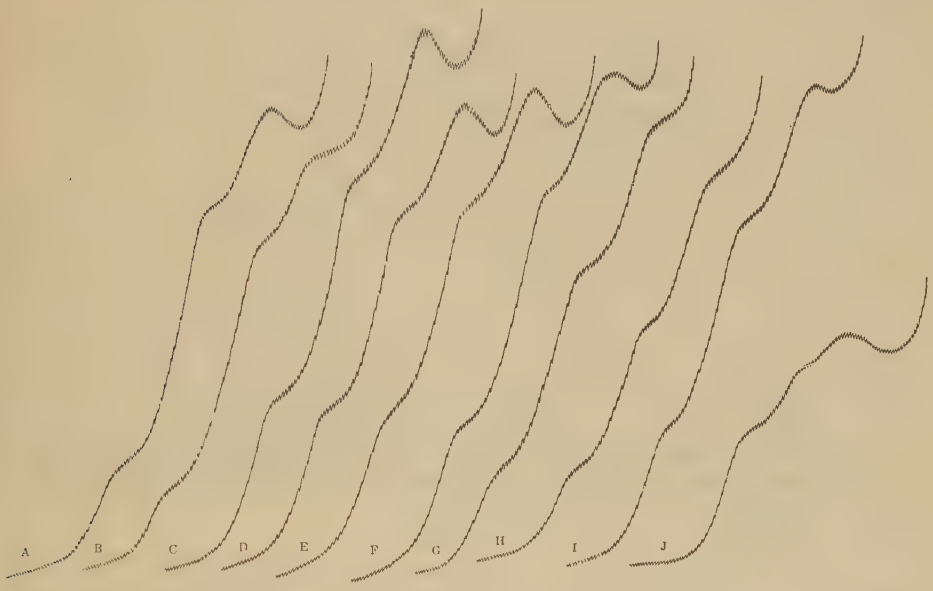


Fig. 10. Polarographic protein wave of meat extractives.
Used ammoniacal cobaltic solution contained trivalent cobalt. Sensitivity, 0.100 μA/mm.

A, *Thunnus thynnus*; B, *Cololabis saira*; C, *Limanda yokohamae*; D, *Sillago sihama*; E, *Ostrea gigas*; F, *Meretrix meretrix lusoria*; G, *Ommatostrephes soloani pacificus*; H, *Neptunus trituberculates*; I, *Penaeus orientalis*; J, drip of frozen whale meat.

saira, and the other which was produced double protein wave such as *Sillago sihama*, *paralichthys olivaceus*, mollusca and crustaceae.

It is considered that in all cases the water soluble protein contained in meat essentially showed double protein wave in ammoniacal cobaltic (II) solution, because all of them showed double protein wave in ammoniacal cobaltic (III) solution. It seems that the single wave of reddy meat which is produced in ammoniacal cobaltic (II) solution may be caused by an interfering substance in meat extractives other than protein, and by this interference the waves are changed from double wave to single wave.

2) Comparative observation of reddy meat and white meat.

In ammoniacal cobaltic (II) solution, water extractives of meat produce a different protein wave in the different species as described above, so in this chapter we examined to make clear a cause of the different protein waves in the species of aquatic animals.

For this experiment, water extractives of meat of *Thunnus thynnus* and *Sillago sihama* were prepared. The one is a sample of which is produced double protein wave and the other is a single protein wave.

As shown in Fig. 11, each extractives of them produced double protein waves or a single protein wave, but when the both extractives added together a single protein wave was observed. So it is sure that the meat extractives of

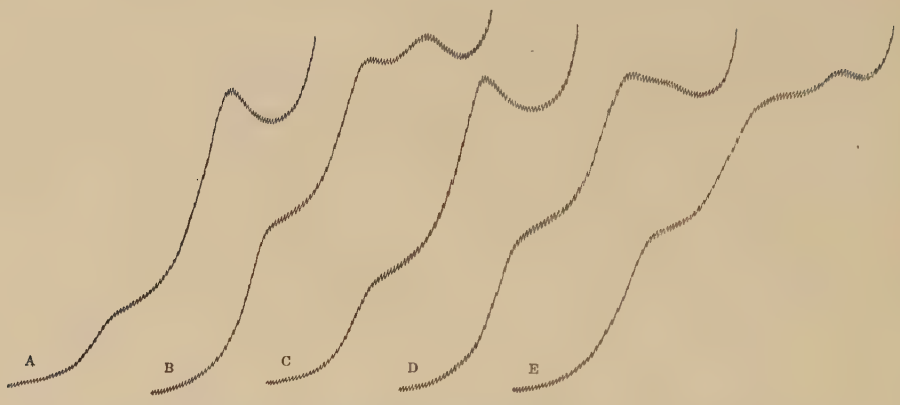


Fig. 11. Comparison of white meat and reddy meat extractives.
Used ammoniacal cobaltic soln. contained divalent cobalt. Sensitivity, 0.100 μA/mm.
A, *Thunnus thynnus* (LINNÉ); B, *Sillago sihama* (FORSKÅL); C, A+B; D, 80% alcoholic extractives of A+B; E, 80% alcohol+B.

Thunnus thynnus contained a certain substance which is affected to the double protein waves.

The same result was obtained to meat extractives of *Sillago sihama* added with alcoholic extractives of *Thunnus thynnus*. In alcohol extractives of meat, amino acid and other low-molecular substance are involved. The substance which is affected to double protein waves seems to a certain amino acid or a low-molecular substance. The details of the substance which is affected to protein wave are examined in latter chapter.

3) Polarographic characteristics of meat for several aquatic animals.

In the former chapter 15 kinds of aquatic animals are compared with the protein wave in a limited condition. Here, polarographic characteristics of meat extractives in details for 15 kinds of aquatic animals are examined. Materials are the same as noted in the former chapter. These raw meats were extracted with water and polarographed by the addition of ammoniacal cobaltic (II) or (III) solution in several concentration. These results are presented in Figs. 12–20, and the relation between concentration of protein and height of protein wave are shown in Figs. 21–26.

In all cases, with increasing concentration of protein the height of the protein wave increased first proportionally to protein concentration but tended finally to a plateau, i.e., a maximal value.

This tendency is the same as reported by TROPP[40] on pure protein.

It is often observed in pure protein that the second wave of double protein wave produce lower than the first wave in the low concentration of the protein, and with increasing concentration the second wave produce higher than the first wave, that is called crossing effect[40] of protein wave.

In this experiment, such a crossing effect could not found, that is, the second wave was always higher than the first wave.

Fig. 12. Polarograms of water extractives of meat—*Limanda yokohamae*.

The ratio of extractives and basic solution is: A, F, 0.25 ml.+20 ml.; B, G, 0.50 ml.+20 ml.; C, H, 1.0 ml. +20 ml.; D, I, 1.5 ml.+20 ml.; E, J, 2.0 ml.+20 ml.; A–E, ammoniacal cobaltic (II) solution; F–J, ammoniacal cobaltic (III) solution. Sensitivity, 0.100 μA/mm.

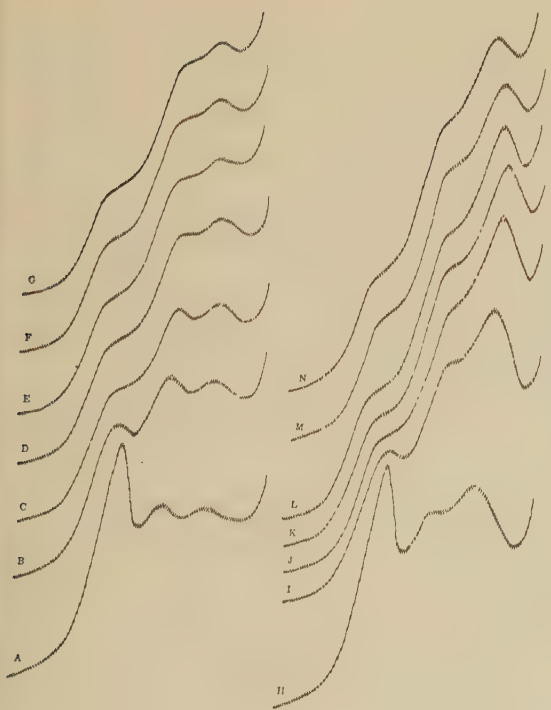


Fig. 13. Polarograms of water extractives of meat—*Sillago sihama*.

The ratio of extractives and basic solution is: A, H, 0.10 ml.+20 ml.; B, I, 0.25 ml.+20 ml.; C, J, 0.50 ml. +20ml.; D, K, 1.0ml.+20ml.; E, L, 1.5ml.+20ml.; F, M, 2.0ml.+20ml.; G, N, 3.0 ml.+20 ml. A–G, ammoniacal cobaltic (II) solution; H–N, ammoniacal cobaltic (III) solution. Sensitivity, 0.100 μA/mm.

Fig. 14. Polarograms of water extractives of meat—*Doryteus bleekeri*.

The ratio of extractives and basic solution is: A, H, 0.10 ml.+20 ml.; B, I, 0.30 ml.+20 ml.; C, J, 0.50 ml.+20ml; D, K, 1.0ml.+20ml.; E, L, 1.5ml.+20 ml.; F, M, 2.0 ml.+20 ml.; G, N, 3.0 ml.+20 ml. A–G, ammoniacal cobaltic (II) solution; H–N, ammoniacal cobaltic (III) solution. Sensitivity, 0.100 μA/mm.

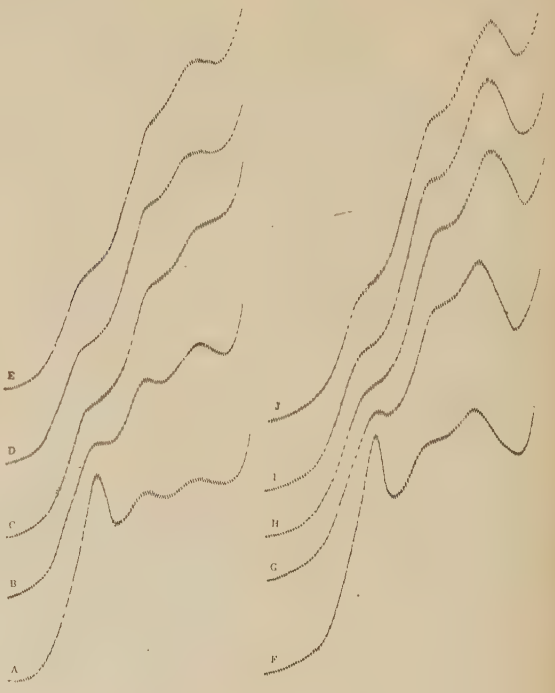


Fig. 15. Polarograms of water extractives of meat — *Ostrea* (*Crassostrea*) *gigas*.

The ratio of extractives and basic solution is: A, F, 0.25 ml.+20 ml;. B, G, 0.5ml.+20ml.; C, H, 10.0ml.+20ml.; D, I, 1.5ml.+20ml.; E, J, 2.0ml.+20ml. A–E, ammoniacal cobaltic (II) solution; F–J, ammoniacal cobaltic (III) solution. Sensitivity, 0.100 μA/mm.

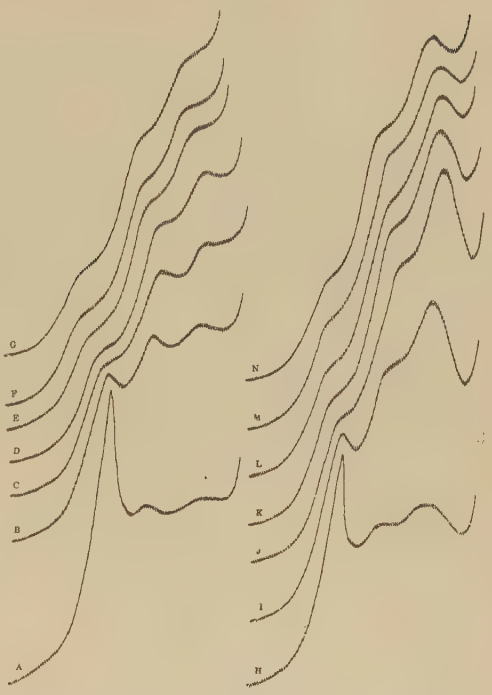


Fig. 16. Polarograms of water extractives of meat—*Meretrix meretrix lusoria*.

The ratio of extractives and basic solution is: A, H, 0.10 ml.+20 ml.; B, I, 0.25ml.+20 ml.; C, J, 0.50 ml.+20 ml.; D, K, 1.0 ml.+20 ml.; E, L, 15 ml.+20 ml.; F, M, 2.0 ml.+20 ml; G, N, 3.0 ml.+20 ml. A–G, ammoniacal cobaltic (II) solution; H–N, ammoniacal cobaltic (III) solution. Sensitivity, 0.100 μA/mm.

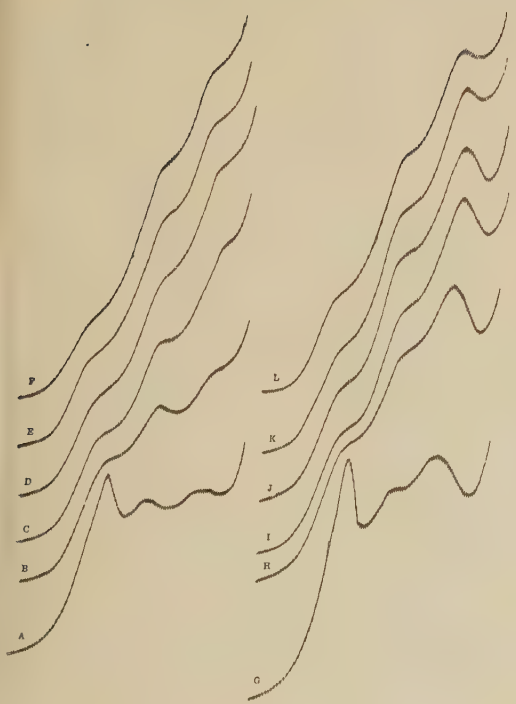


Fig. 17. Polarograms of water extractives of meat—*Anadara* (*Scaplarca*) *inflata*.

The relation of extractives and basic solution is: A, G, 0.25ml.+20ml.; B, H, 0.5ml.+20ml.; C, H, 1.0ml.+20ml.; D, I, 1.5ml.+20ml.; E, J, 2.0ml.+20ml.; F, L, 3.0ml.+20ml. A–J, ammoniacal cobaltic (II) solution; G–L, ammoniacal cobaltic (III) solution. Sensitivity, 0.100 μA/mm.

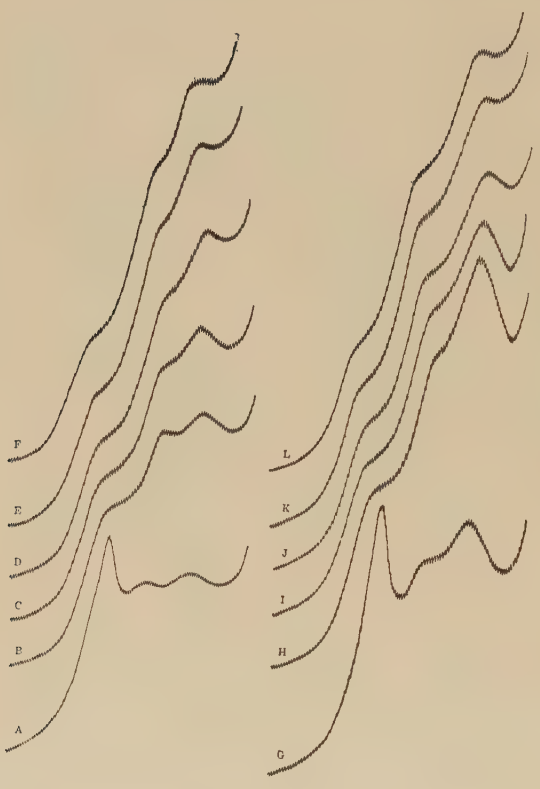


Fig. 18. Polarograms of water extractives of meat — *Penaeus orientalis*.

The ratio of extractives and basic solution is: A, G, 0.10ml.+20ml.; B, H, 0.25ml.+20ml.; C, I, 0.50 ml. +20ml.; D, J, 1.0ml.+20ml.; E, K, 1.5ml.+20ml.; F, L, 2.0ml.+20ml. A–F, ammoniacal cobaltic (II) solution; G–L, ammoniacal cobaltic (III) solution. Sensitivity, 0.100 μA/mm.

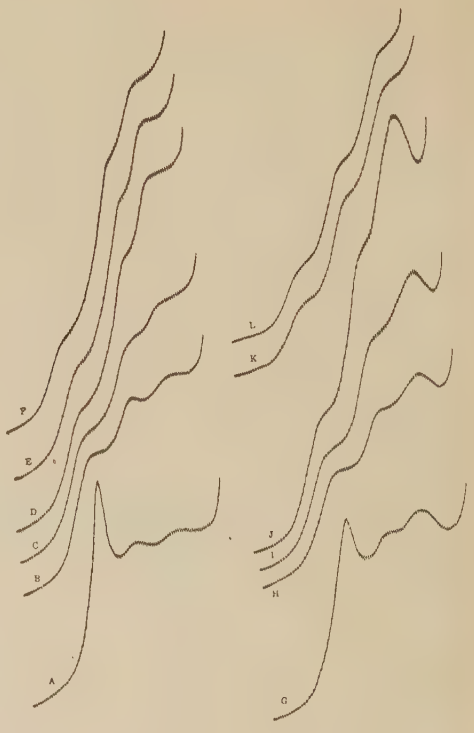


Fig. 19. Polarograms of water extractives of meat—*Neptunus trituberculates*.

The ratio of extractives and basic solution is: A, G, 0.10ml.+20ml.; B, H, 0.25ml. +20ml.; C, I, 0.50ml.+20ml.; D, J, 1.0ml. +20ml.; E, K, 1.5ml.+20ml.; F, L, 2.0ml. +20 ml. A–F, ammoniacal cobaltic (II) solution, G–L, ammoniacal cobaltic (III) solution. Sensitivity, 0.100 μA/mm.

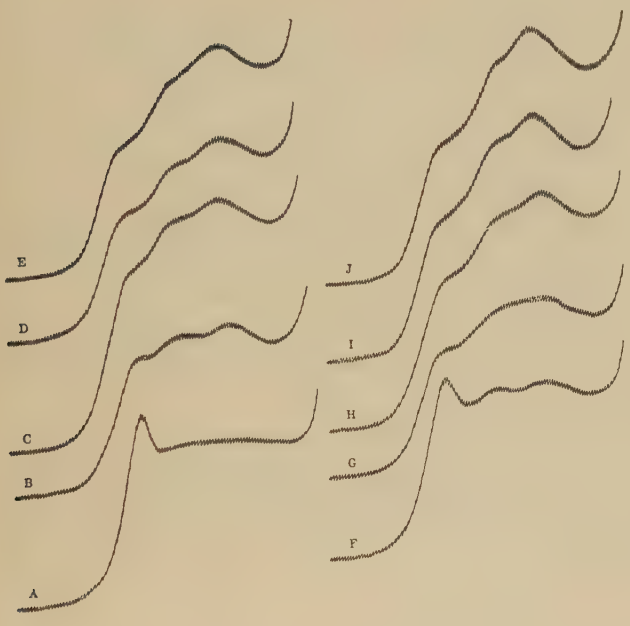
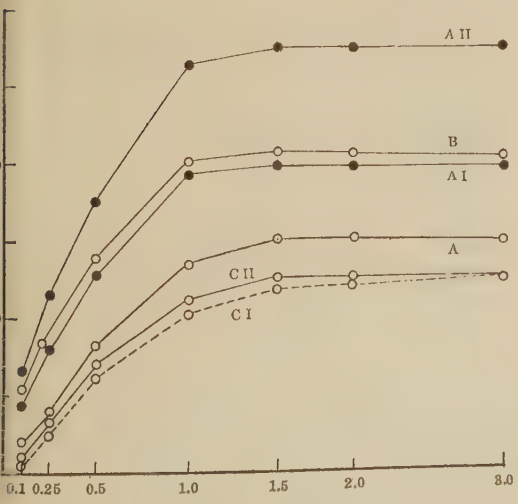


Fig. 20. Polarograms of drip of frozen whale meat.

The ratio of drip (diluted with water 50 times) and basic solution is: A, F, 0.20 ml. + 20 ml.; B, G, 0.50 ml. +20 ml.; C, H, 1.0 ml. +20 ml.; D, I, 2.0 ml. +20 ml.; E, J, 3.0 ml. + 20 ml. A–E, ammoniacal cobaltic (II) solution; F–J, ammoniacal cobaltic (III) solution. Sensitivity, 0.100 μA/mm.

In all cases extractives of meat produced double protein wave in ammoniacal cobaltic (III) solution, and the waves were heigher than the protein wave in ammoniacal cobaltic (II) solution.



ig. 21. A, *Cololabis saira* (BREVOORT); B, *Katsuwonus pelamis* (LINNÉ); C, *Cyprinus caprio* (LINNÉ).

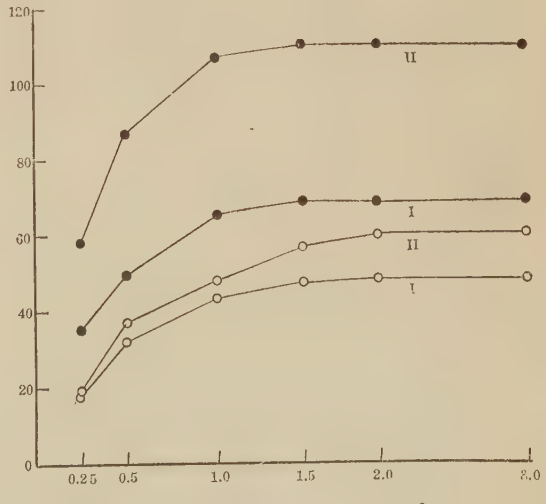


Fig. 22. *Sillago sihama* (FORSKÅL).

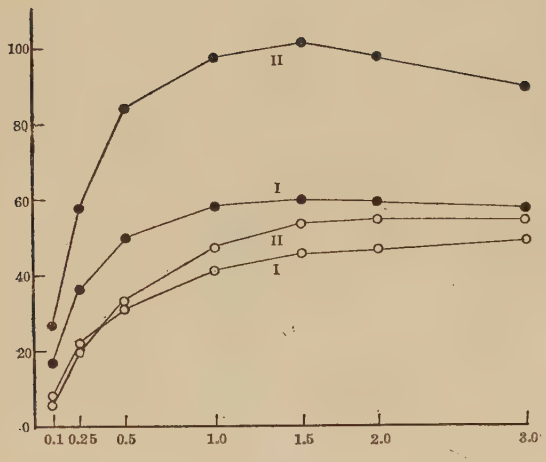


Fig. 23. *Limanda yokohamae* (GÜNTHER)

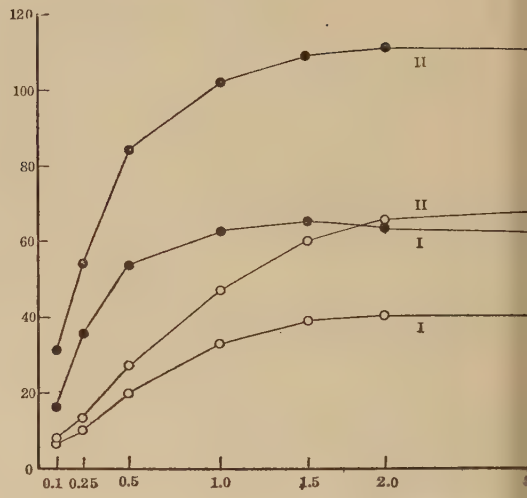


Fig. 24. *Doryteus bleekeri* (KEFERSTEIN)

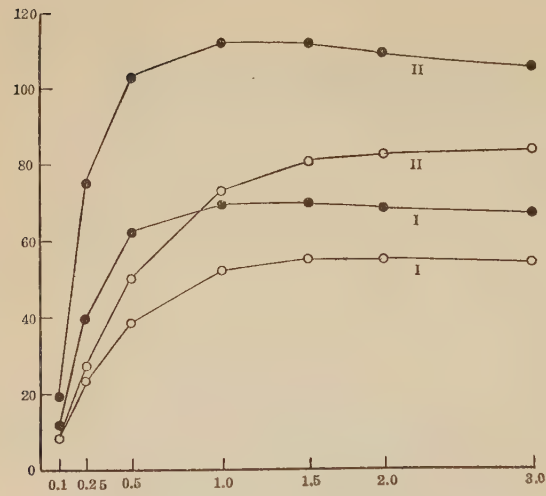


Fig. 25. *penaeus orientalis* KISHINOUGE

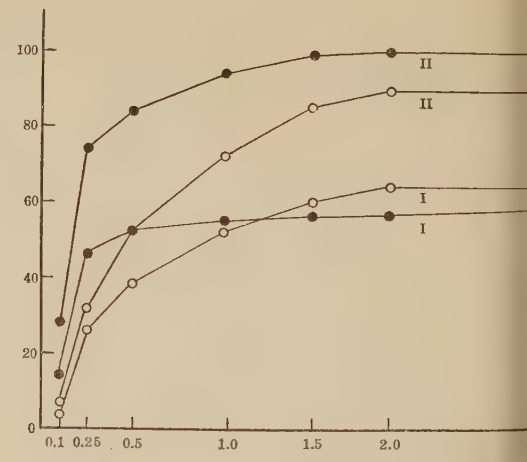


Fig. 26. *Meretrix meretrix lusoria* (GMELIN)

Figs. 21-26. Relation between concentration of meat extractives and protein wave height. Ordinary—wave height (mm); abscissa—relative concentration of meat extractives (*ml.* of water extractives of meat added to ammoniacal cobaltic solution).

I, first wave; II, second wave; —●—, ammoniacal cobaltic solution containing trivalent cobalt; —○—, the soln, containing divalent cobalt.

5. Comparative studies of common muscle and dark colored muscle by polarographic protein wave

1) Comparative observation of several common muscle and dark colored muscle.

Fresh meat of *Katsuwonus pelamis*, *Cololabis saira*, *Thunnus thynnus* and *Scomber japonicus* was separated into common muscle and dark colored muscle and each of them extracted with water, and the filtrates were used for the test solution. For basic solution ammoniacal cobaltic (II) and (III) solutions were used. These polarograms and the relation between the concentration and height of protein wave are as shown in Figs. 27–32 and Table 3.

Protein wave of dark colored muscle in ammoniacal cobaltic (II) solution produce double protein wave in low concentration of protein and single wave in higher concentration. On the other hand all common muscle produce single protein wave. In higher concentration of protein both muscle produce single protein wave, but the shape of the waves are somewhat difference in each other. That is, common muscle produce single protein wave having a maximal shape and dark colored muscle does not show the maximal shape.

In ammoniacal cobaltic (III) solution both muscle samely produce double

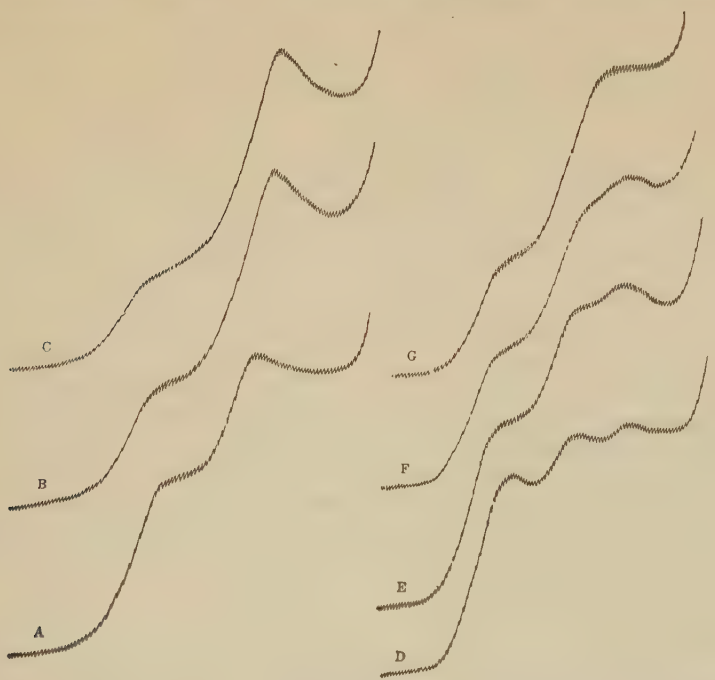


Fig. 27. Comparison of common muscle and dark colored muscle—*Katsuwonus pelamis*.

A–C, Common muscle; D–G, dark colored muscle. The ratio of meat extractives (extracted 10 times of water) and ammoniacal cobaltic (II) solution, D, 0.10 ml.+20 ml.; A, E, 0.20 ml.+20 ml.; F, 0.50 ml.+20 ml.; C, G, 1.0 ml.+20 ml. Sensitivity, 0.100 μA/mm.

Fig. 28. Comparison of common muscle and dark colored muscle—*Cololabis saira*-(A).

A–H, common muscle; I–P, dark colored muscle. The ratio of meat extractives (extracted 10 times of water) and ammoniacal cobaltic (II) solution. A, I, 0.10 ml.+20 ml.; B, J, 0.20 ml.+20 ml.; C, K, 0.25 ml.+20 ml.; D, L, 0.50 ml.+20 ml.; E, M, 1.0ml.+20ml.; F, N, 1.5ml.+20ml.; G, O, 2.0ml.+20ml.; H, P, 3.0ml.+20 ml. Sensitivity, 0.100 μA/mm.

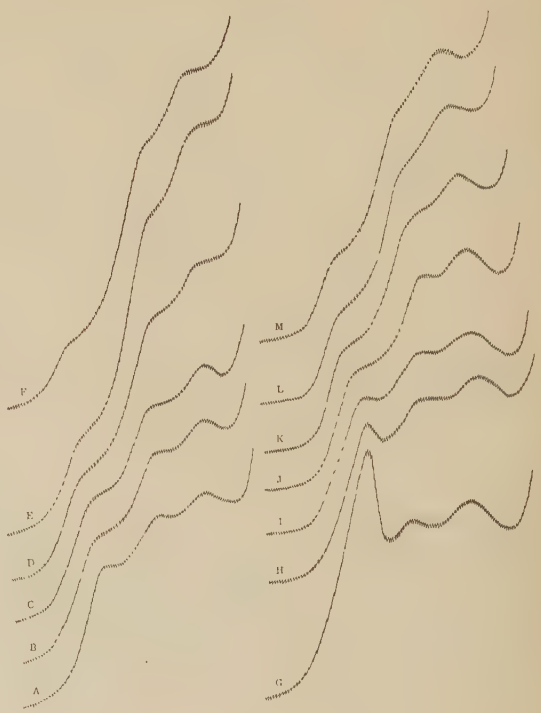


Fig. 29. Comparison of common muscle and dark colored muscle—*Colobabis saira*-(B).

A–F, common muscle; G–M, dark colored muscle. The ratio of meat extractives (extracted 10 times of water) and ammoniacal cobaltic (III) solution, A, G, 0.10 ml.+20 ml.; B, H, 0.20 ml.+20 ml.; C, I, 0.25 ml.+20 ml.; D, J, 0.50 ml.+20 ml.; E, K, 1.0 ml.+20 ml.; F, L, 2.0 ml.+20 ml. Sensitivity, 0.100 μA/mm.

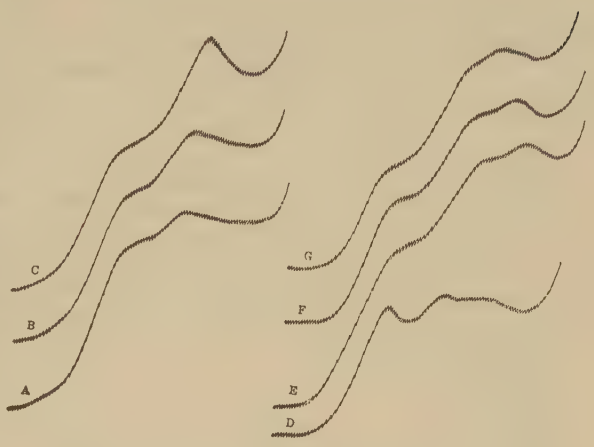


Fig. 30. Comparison of common muscle and dark colored muscle—*Scomber japonicus*.

A–C, common muscle; D–G, dark colored muscle. The ratio of meat extractives (extracted 50 times of water) and ammoniacal cobaltic (II) solution, A, D, 1.0 ml.+20 ml.; B, E, 2.0 ml.+20 ml.; C, F, 3.0 ml.+20 ml.; G, 5.0 ml.+20 ml. Sensitivity, 0.100 μA /mm.

Fig. 31. Comparison of common muscle and dark colored muscle—*Thunnus thynnus* and *Katsuwonus pelamis*.

The ratio of meat extractives (extracted with 10 times of 0.1 M NH_4Cl) and ammoniacal cobaltic solution, 2.0 ml.+20 ml. A, common muscle of *Thunnus thynnus*; B, common muscle of *Katsuwonus pelamis*; C, dark colored muscle of *Thunnus thynnus*; D, dark colored muscle of *Katsuwonus pelamis*. Sensitivity, 0.100 μA/mm.

protein wave, and these is no difference in the shape of protein wave. The relations between concentration of protein and wave height in both muscle are the same as presented in the former chapter, that is, with increasing concentration of protein the height of the protein wave increases first proportionally to protein concentration but tends finally to a plateau, i,e., a maximal value.

The wave height of common muscle is higher than dark colored muscle. This tendency is the same as when the both muscle are extracted with NH_4Cl solution.

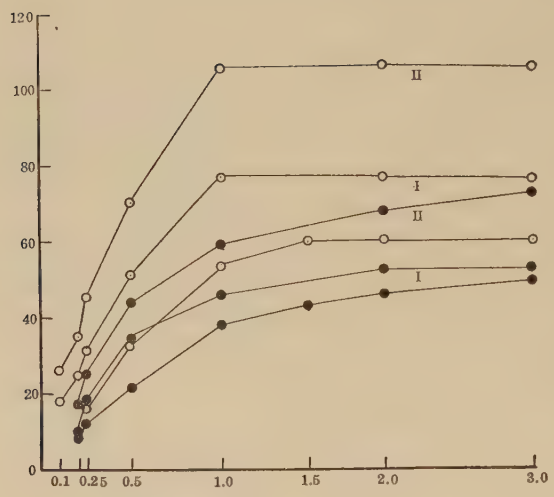


Fig. 32. Relation between concentration of meat extractives and height of protein wave.

Ordinary — wave height (mm); abscissa—relative concentration of meat extractives (ml. of water extractives of meat added to ammoniacal cobaltic solution); I, first wave; II second wave in ammoniacal cobaltic (III) solution; others in ammoniacal cobaltic (II) solution. —○—, common muscle; —●—, dark colored muscle.

Table 3. Comparison of common muscle and dark colored muscle by the protein wave.

C, M, common muscle; D, M, dark colored muscle; I, the first wave; II, the second wave of protein wave; $CoCl_2$, ammoniacal cobaltic (II) solution; luteo, ammoniacal cobaltic (III) solution.

Species of fish	Extract method	Extractive+basic soln.	C, M		D, M	
			I	II	I	II
		ml ml	mm	mm	mm	mm
Cololabis saria	Extracted 10 times of water	1.0+20 ($CoCl_2$)	54	—	39	—
〃	〃	2.0+20 ($CoCl_2$)	60	—	46	—
〃	〃	1.0+20 (luteo)	77	106	46	59
〃	〃	2.0+20 (luteo)	78	110	53	68
Katsuwons pelamis	〃	1.0+20 ($CoCl_2$)	53	—	46	—
〃	Extracted 20 times of NH_4Cl	2.0+20 ($CoCl_2$)	48	—	44.5	—
Thunnus thynnus	〃	2.0+20 ($CoCl_2$)	50	—	37	—
Scomber japonicus	Extracted 50 times of water	1.0+20 ($CoCl_2$)	10	—	6	—
〃	〃	2.0+20 ($CoCl_2$)	26	—	22	26

2) Interference of meat extractives for albumin-protein wave.

In the comparative experiments among several aquatic animals and between common muscle and dark colored muscle by the protein wave, we found a certain substance to interfered the shape of double protein wave.

Here it makes to clear the interference to double protein wave.

In this experiment 0.2% of albumin solution was used for the material which produced double protein wave, and examined the change of the waves by the

addition of extractives of common and dark colored muscle. Polarograms of albumin solution by the addition of meat extractives are shown in Figs. 33–37, and the change of wave height is presented in Table 4.

Fig. 33. Polarograms of albumin solution by the addition of meat extractives.

E, extractives of common muscle of *Katsuwonus pelamis*, (extracted 20 times quantity of 1 M NH_4Cl); basic soln., ammoniacal cobaltic (II) solution.

	0.2% albumin soln.	E	Water	Basic soln.
A	2.0 ml.	0 ml.	2.0 ml.	20 ml.
B	2.0	0.1	1.9	20
C	2.0	0.2	1.2	20
D	2.0	2.0	0	20

Sensitivity, 0.100 μA/mm.

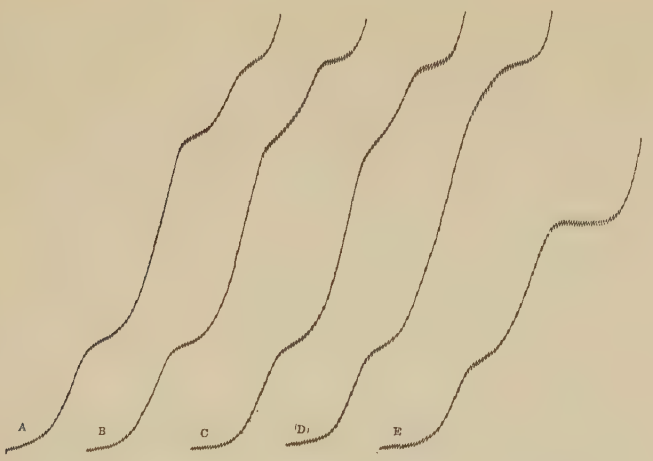


Fig. 34. Polarograms of albumin solution by the addition of meat extractives.

E, extractives of dark colored muscle of *Katsuwonus pelamis*, (extracted 20 times quantity of 1 M NH_4Cl); basic solns., ammoniacal cobaltic (II) solution.

	0.2% albumin soln.	E	water	basic soln.
A	2.0 ml.	0 ml.	2.0 ml.	20 ml.
B	2.0	0.2	1.8	20
C	2.0	0.5	1.5	20
D	2.0	0.7	1.3	20
E	2.0	2.0	0	20

Sensitivity, 0.100 μA/mm

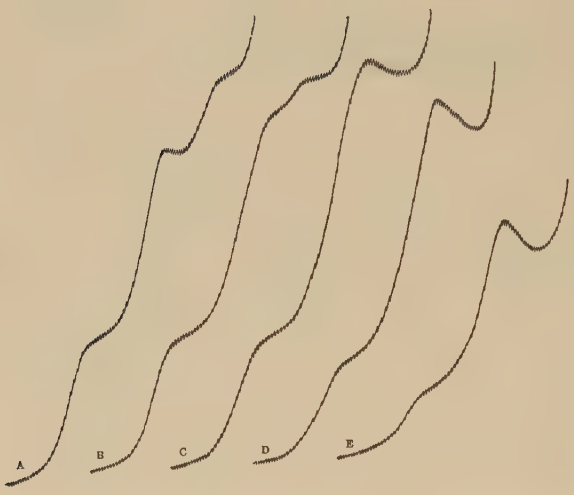


Fig. 35. Polarograms of albumin solution by the addition of meat extractives.

E., extractives of common muscle of *Cololabis saira*, (extracted 10 times of water), basic soln., ammoniacal cobaltic (II) solution.

	0.2% albumin soln.	E	Water	Basic soln.
A	2.0 ml.	0 ml.	2.0 ml.	20 ml.
B	2.0	0.1	1.9	20
C	2.0	0.3	1.7	20
D	2.0	0.5	1.5	20
E	2.0	2.0	0	20

Sensitivity, 0.100 μA/mm.

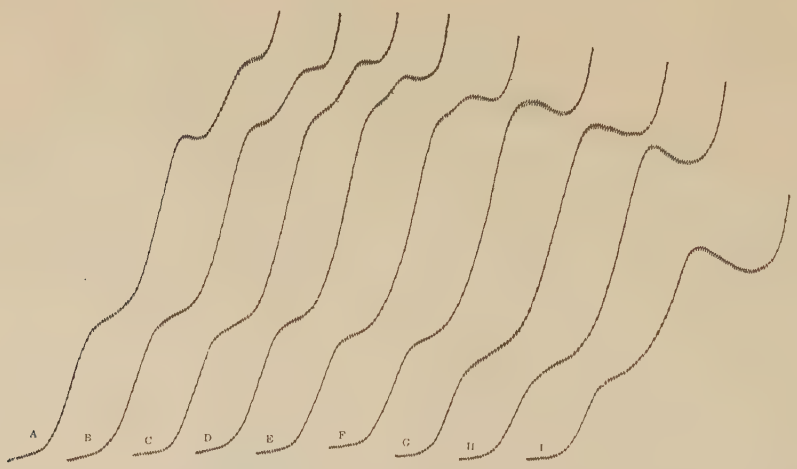


Fig. 36. Polarograms of albumin solution by the addition of meat extractives.

E., extractives of dark colored muscle of *Cololabis saira*, (extracted 10 times of water), basic soln., ammoniacal cobaltic (II) solution.

	0.2% albumin soln.	E	Water	Basic soln.
A	2.0 ml.	0 ml.	2.0 ml.	20 ml.
B	2.0	0.1	1.9	20
C	2.0	0.2	1.8	20
D	2.0	0.3	1.7	20
E	2.0	0.4	1.6	20
F	2.0	0.7	1.3	20
G	2.0	1.0	1.0	20
H	2.0	1.5	0.5	20
I	2.0	2.0	0	20

Sensitivity, 0.100 μA/mm.

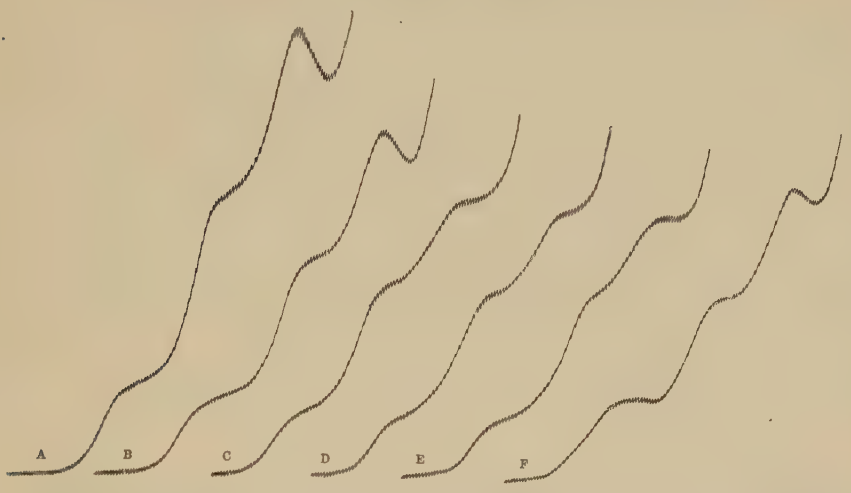


Fig. 37. Polarograms of albumin solution by the addition of meat extractives. E., extractives of dark colored muscle of *Cololabis saira*, (extracted 10 times of water), basic soln., ammoniacal cobaltic (II) solution.

	0.2% albumin soln.	E	Water	Basic soln.
A	1.0 ml.	0 ml.	2.0 ml.	20 ml.
B	1.0	0.5	1.5	20
C	1.0	1.0	1.0	20
D	1.0	1.5	0.5	20
E	1.0	2.0	0	20
F	0	2.0	2.0	20

Sensitivity, 0.100 μA/mm.

Table 4. Influence of meat extractives to protein wave of albumin. I, the first wave of protein wave; II, the second wave of protein wave; $CoCl_2$, ammoniacal cobaltic (II) solution; luteo salt, ammoniacal cobaltic (III) solution.

Kind of extractives	Kind of basic solution											
		0.2% albumin soln. (ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0
		extractives (ml)	0	0.1	0.2	0.3	0.4	0.5	1.0	1.0	1.5	2.0	2.0
		water (ml)	2.0	1.9	1.8	1.7	1.6	1.5	1.0	1.0	0.5	0	2.0
		basic soln. (ml)	20	20	20	20	20	20	20	20	20	20	20
Extractives of *Katsuwonus* pelamis meat, 1 M NH_4Cl	$CoCl_2$	I	72	88	95	—	—	—	—	—	—	50	50
		II	96	94	98	—	—	—	—	—	—		
Ditto, dark colored muscle	$CoCl_2$	I	72	71	—	—	—	70	98	—	—	45	45
		II	96	98	—	—	—	97					
Water extractives of common muscle—*Cololabis saira*	$CoCl_2$	I	68	80	—	92	—	89	—	—	—	57	56
		II	95	92	—								
Ditto, dark colored muscle	$CoCl_2$	I	68	72	74	75	76	—	83	81	79	47	47
		II	95	87	88	86	83	—					
Ditto	luteo salt	I	32	—	—	—	—	55.5	—	41	37.5	37.5	38
		II	65	—	—	—	—	108.5	—	78	62.5	60	60

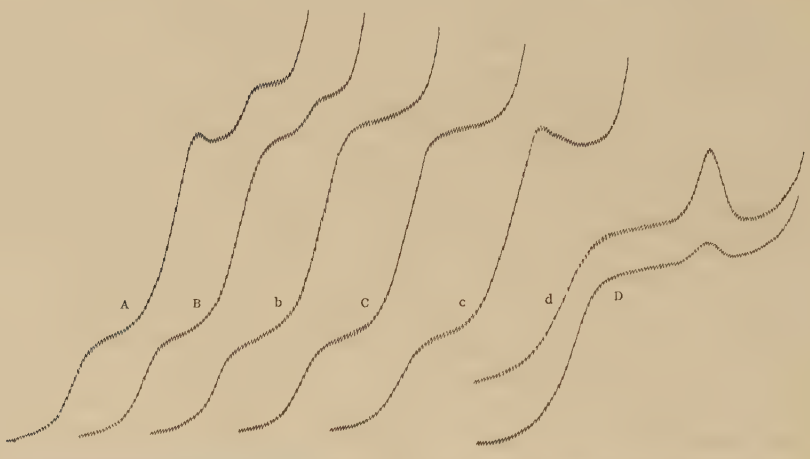


Fig. 38. Interference of alcoholic extractives of meat to protein wave—*Cololabis saira*.

A, 2.0 ml of 0.2% albumin solution+20 ml of ammoniacal cobaltic (II) solution; B, A+0.5 ml of alcoholic extractives of dark colored muscle; b, A+0.5 ml of alcoholic extractives of common muscle; C, A+1.0 ml of alcoholic extractives of dark colored muscle; c, A+1.0 ml of alcoholic extractives of common muscle; D, 2.0 ml of alcoholic extractives of dark colored muscle+ammoniacal cobaltic (II) solution; d, 2.0 ml of alcoholic extractives of common muscle+ammoniacal cobaltic (II) solution. Sensitivity, 0.100 μA/mm.

Table 5. Change of protein wave by the addition of alcoholic extractives of meat. II, double protein wave; I, single protein wave; –, +, ++, +++, degree of peculiar wave (*cf.* Fig. 38, d, D); Extractives, 80% alcoholic extractives of fish meat; C. M., common muscle; D. M., dark colored muscle; basic soln. ammoniacal cobaltic (II) solution.

0.2% albumin soln. (ml)	2.0	2.0	2.0	2.0	2.0	0
Extractives (ml)	0	0.1	0.3	0.5	1.0	2.0
Water (ml)	2.0	1.9	1.7	1.5	1.0	2.0
Basic soln. (ml)	20	20	20	20	20	20
Species						
Katsuwonus pelamis C. M.	II	±	I	I	I	+++
〃 D. M.	II	II	±	I	I	+
Thunnus thynnus, C. M.	II	II	±	I	I	++
〃 D. M.	II	II	II	II	I	+
Cololabis saira C. M.	II	II	±	I	I	++
〃 D. M.	II	II	II	II	I	+
Sillago sihama	II	II	II	II	II	–
Paralichthys clivaceus	II	II	II	II	II	–
Meretrix meretrix lusoria	II	II	II	II	II	–
Neptunus trituberculates	II	II	II	II	II	–

In all cases double protein wave of albumin solution changed to single protein wave by the addition of meat extractives. Water extractives of common muscle are more interfered than the extractives of dark colored muscle. In the addition of meat extractives the increase of wave height is supposed, but actually the wave height decreased, so it is sure that the water extractives of meat involve a certain substance which interfered to the double protein wave.

The same experiment for alcoholic extractives of meat were also made. The results are presented in Fig. 38 and Table 5. Alcoholic extractives of white meat such as *Sillago sihama* and *Paralichthys clivaceus* and meat extractives of *Meretrix meretrix lusoria* and *Neptunnus trituberculates* are not interefered to the double protein wave, but the extractives of reddy meat such as *Katsuwonus pelamis* and *Thunnus thynnus* are interfered the double protein wave. Especially in common muscle of the reddy meat such as *Thunnus thynnus*, the interference is more distinct than in dark colored muscle.

Therefore, it is sure that the interference of protein wave in water extractives of meat due to a certain substance containing in alcoholic extractives of reddy fish meat.

The interfere substance produce a peculiar wave in itself in ammoniacal cobaltic (II) solution. It is supposed that the substance interfere in a certain concentration in protein solution and in high concentration the substance produce a peculiar wave.

3) Substance to interfere the protein wave.

It was already clarified that the interfering substance which was affected to double protein wave existed in water extractives of reddy meat. In this chapter we examine the substance in details.

First of all we observed the contents of amino acids in alcoholic extractives of several kinds of meat quated in other reports[41-43]. The contents of amino acids in aquatic animals are presented in Table 6.

In reddy meat of fish such as *Katsuwonus pelamis* and *Cololabis saira*, contents of histidine[45] are numerous and glycine, lysine, alanine, and glutamic acid are second. In white meat such as *Kareius bicoloratus* and *Mylio macrocephalus*, the contents of glycine, alanine, asparatic acid, glutamic acid and lysine are higher than the others. Cuttle fish meat contents proline in the first and arginine, glycine and alanine in the second, and histidine is variant in the species. In shell fish glycine, alanine, arginine asparatic acid and glutamic acid are main and the others are proline, serine, alanine and glutamic acid. Reddy fish meat contain more quantity of histidine[45] than white meat, and in common muscle of the reddy meat the contents of histidine, asparatic acid, arginine, phenylalanine, creatine and creatinine are notable. It seems that the different polarographic wave in the species and in the different part of muscle of aquatic animals relate to the component of amino acid containing in water extractives of meat.

In this chapter we examine the interference of amino acids to the protein wave. Four kinds of amino acids were used for this experiment, i.e., histidine, arginine, tryptophane and phenylalanine which is interfered to cystine wave[46]. For the material to produce double protein wave 0.2% of albumin solution was used.

Table 6. Contents of amino acids in alcoholic extractives of aquatic animals. (mg %)[41-44]
C. M., common muscle; D. M., dark colored muscle.

Species of aquatic animal / amino acid	*Katsuwonus pelamis*		*Scomber japonicus*		*Trachurus japonicus*		*Cyprinus capoio*	*Kareius bicoloratus*	*Mylio macroceph*	*Ommatostrephes solani pacificus*	*Sepia esculenta*	*Penaeus japonicus*	*Ostrea gigas*
	C. M.	D. M.	C. M.	D. M.	C. M.	D. M.							
Glycine	7.0	8.4	54	53	40	37	163.6	33.6	97	42	104	1250	340
Alanine	10.9	22	37	39	28	23	28.0	44.6	27	75	179	58	366
Valine	39	35	14	13	18	13	4.6	3.3	5.1	20	26	19	10.5
Leucine	3.3	3.3	14	16	20	20	7.5	1.9	8.5	18	23	17	9.6
Isoleucine	1.4	1.4	10	9.6	8.3	8.1	42	3.3	7.3	15	12	11	4.9
Serine	2.7	2.9	6.9	9.2	7.1	7.4	—	10.8	3.9	16	112	108	3.0
Threonine	4.0	6.0	10	9.0	9.0	7.1	—	14.9	13	23	53	15	—
Methionine	2.7	2.1	7	6.3	4.3	3.7	6.4	0.7	1.1	12	31	11	3.4
Asparatic acid	8.0	2.4	9.8	6.3	12	2.5	2.9	2.6	17	trace	29	trace	38.5
Glutamic acid	12.2	8.4	20	21	19	18	11.6	6.7	19	26	44	65	148
Tyrosine	3.5	2.5	7	6.0	5.3	4.6	4.6	1.1	1.1	4	8.4	1	7.6
Phenylalanine	2.3	1.8	9	9.6	13	3.1	2.4	1.0	11	6	9.3	7	6.0
Proline	7.8	4.6	5.4	5.3	8.4	7.9	4.0	5.3	3.9	479	379	230	8.0
Tryptophane	1.2	0.7	3	2.8	1.6	1.4	—	0.3	2.0	1.4	1.6	0.9	1.5
Arginine	1.7	0.7	6.1	5.7	6.0	2.3	7.5	5.2	3.0	99	280	686	53.5
Lysine	10.3	18	22	22	30	23	23.7	14.5	13	9	32	15	3.1
Histidine	741.6	148	563	296	163	69	67.9	2.8	5.4	140	12	17	—

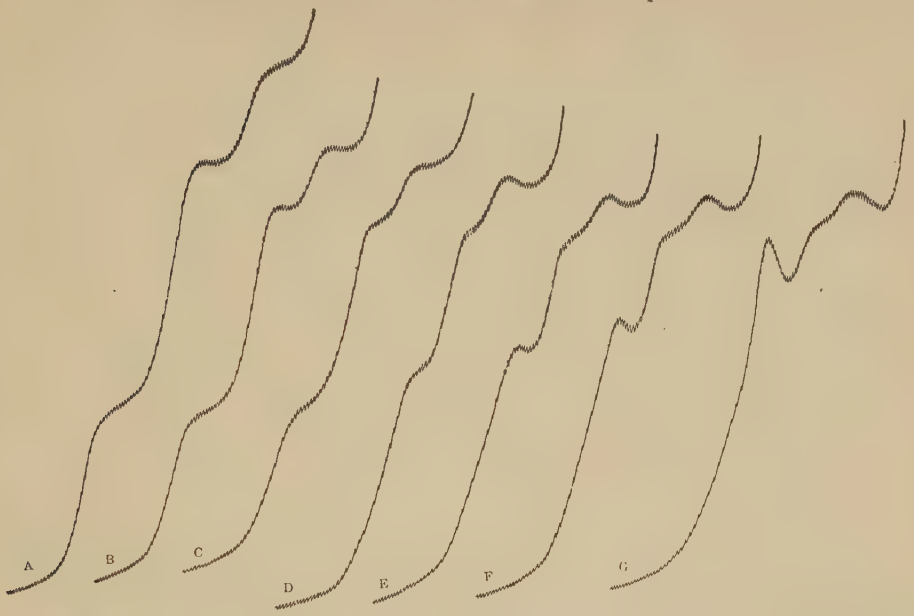


Fig. 39. Interference of arginine solution to the protein wave. Basic solution is ammoniacal cobaltic (II) solution.

	0.2% albumin soln.	1% arginine soln.	Water	Basic soln.
A	2.0 ml.	0 ml.	2.0 ml.	20 ml.
B	2.0	0.1	1.9	20
C	2.0	0.3	1.7	20
D	2.0	0.5	1.5	20
E	2.0	0.6	1.4	20
F	2.0	0.7	1.3	20
G	2.0	1.0	1.0	20

Sensitivity, 0.100 μA/mm.

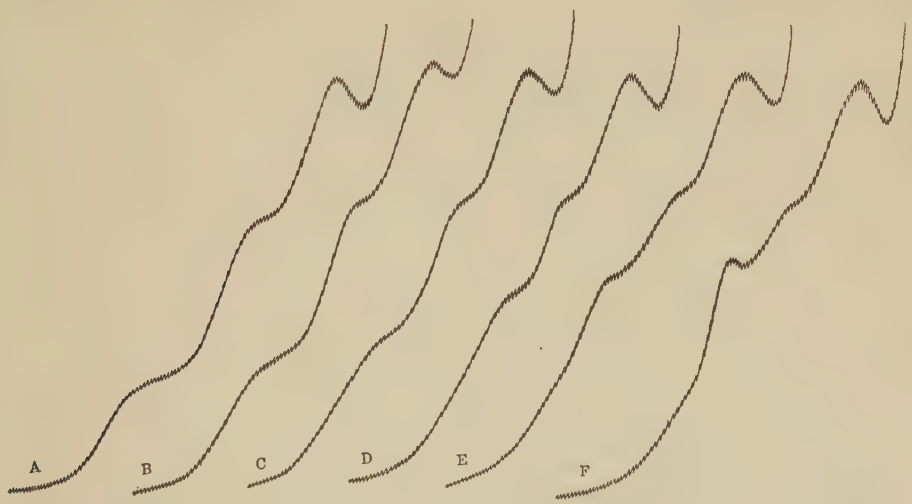


Fig. 40. Interference of arginine solution to the protein wave. Basic soln. is ammoniacal cobaltic (III) solution.

	0.2% albumin soln.	1% arginine soln.	Water	Basic soln.
A	2.0 ml.	0 ml.	2.0 ml.	20 ml.
B	2.0	0.1	1.9	20
C	2.0	0.2	1.8	20
D	2.0	0.3	1.7	20
E	2.0	0.4	1.6	20
F	2.0	0.5	1.5	20

Sensitivity, 0.100 μA/mm.

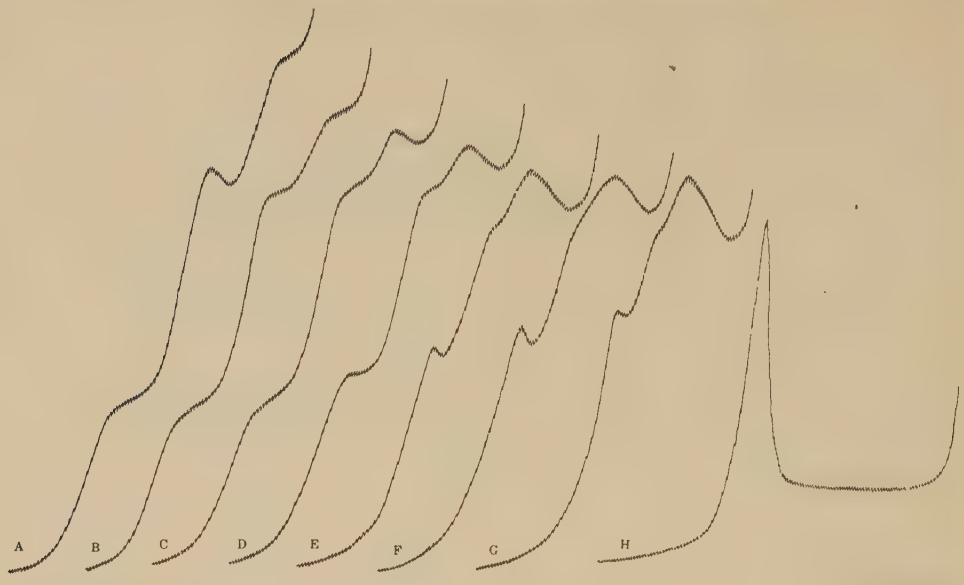


Fig. 41. Interference of phenylalanine to the protein wave. Basic soln. is ammoniacal cobaltic (II) solution.

	0.2% albumin soln.	1% phenylalanine soln.	Water	Basic soln.
A	2.0 ml.	0 ml.	2.0 ml.	20 ml.
B	2.0	0.1	1.9	20
C	2.0	0.25	1.75	20
D	2.0	0.5	1.5	20
E	2.0	1.0	1.0	20
F	2.0	1.5	0.5	20
G	2.0	2.0	0	20
H	0	2.0	2.0	20

Sensitivity, 0.100 μA/mm.

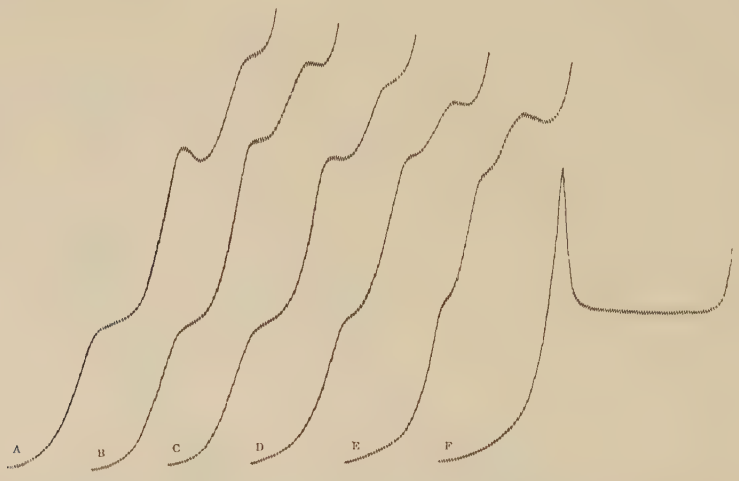


Fig. 42. Interference of tryptophane to the protein wave. Basic soln. is ammoniacal cobaltic (II) solution.

	0.2% albumin soln.	1% tryptophane soln.	Water	Basic soln.
A	2.0 ml.	0 ml.	2.0 ml.	20 ml.
B	2.0	0.1	1.9	20
C	2.0	0.5	1.5	20
D	2.0	1.0	1.0	20
E	2.0	2.0	0	20
F	2.0	2.0	2.0	20

Sensitivity, F, 0.200 μA/mm, Others, 0.100 μA/mm.

Fig. 43. Interference of histidine to the protein wave. Basic soln. is ammoniacal cobaltic (II) solution.

	0.2% albumin soln.	1.0% histidine soln.	Water	Basic soln.
A	2.0 ml.	0 ml.	2.0 ml.	20 ml.
B	2.0	0.05	1.95	20
C	2.0	0.1	1.9	20
D	2.0	0.3	1.7	20
E	2.0	0.5	1.5	20
F	2.0	1.0	1.0	20
G	0	2.0	2.0	20

Sensitivity, 0.100 μA/mm.

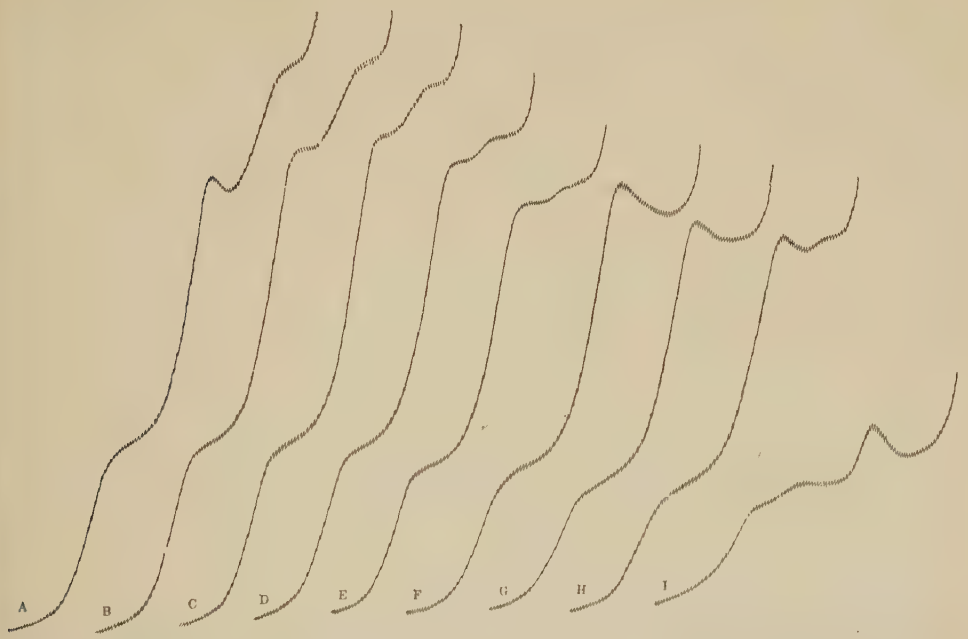


Fig. 44. Interference of histidine to the protein wave. Basic soln. is ammoniacal cobaltic (II) solution.

	0.2% albumin soln.	0.1% histidine soln.	Water	Basic soln.
A	2.0 ml.	0 ml.	2.0 ml.	20 ml.
B	2.0	0.1	1.9	20
C	2.0	0.2	1.8	20
D	2.0	0.3	1.7	20
E	2.0	0.5	1.5	20
F	2.0	1.0	1.0	20
G	2.0	1.5	0.5	20
H	2.0	2.0	0	20
I	0	2.0	2.0	20

Sensitivity, 0.100 μA/mm.

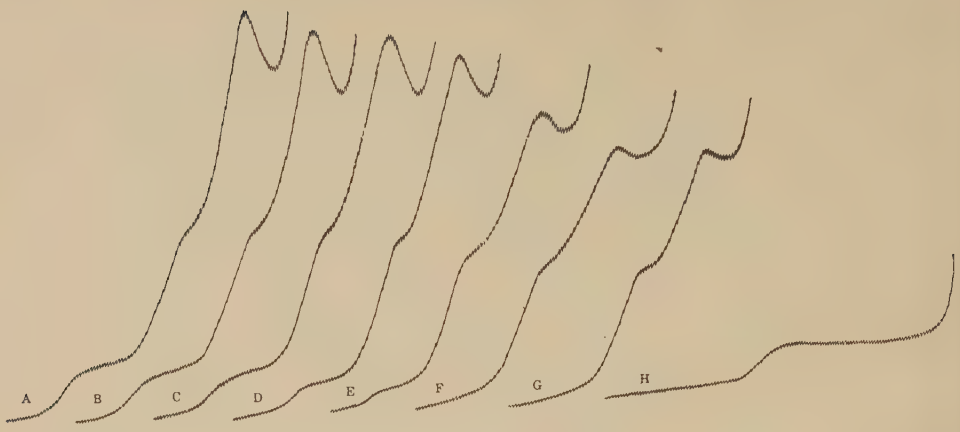


Fig. 45. Interference of histidine to the protein wave. Basic soln. is ammoniacal cobaltic (III) solution.

	0.2% albumin soln.	1.0% histidine soln.	Water	Basic soln.
A	2.0 ml.	0 ml.	2.0 ml.	20 ml.
B	2.0	0.1	1.9	20
C	2.0	0.2	1.8	20
D	2.0	0.5	1.5	20
E	2.0	1.0	1.0	20
F	2.0	1.5	0.5	20
G	2.0	2.0	0	20
H	0	2.0	2.0	20

Sensitivity, 0.400 μA/mm.

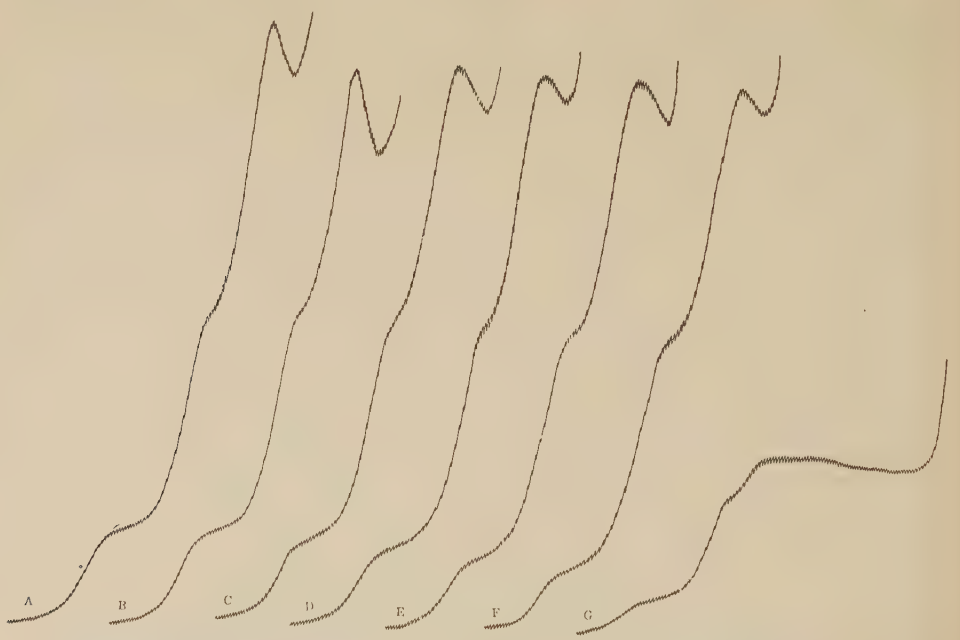


Fig. 46. Interference of histidine to the protein wave. Basic soln. is ammoniacal cobaltic (III) solution.

	0.2% albumin soln.	0.1% histidine soln.	Water	Basic soln.
A	2.0 ml.	0 ml.	2.0 ml.	20 ml.
B	2.0	0.1	1.9	20
C	2.0	0.5	1.5	20
D	2.0	1.0	1.0	20
E	2.0	1.5	0.5	20
F	2.0	2.0	0	20
G	0	2.0	2.0	20

Sensitivity, 0.200 μA/mm.

max., maximum of cobalt, showed no protein wave; $CoCl_2$, ammoniacal cobaltic (II) solution; luteo salt, ammoniacal cobaltic (III) solution; I, the first wave of protein waves; II, the second wave of protein waves.

Species and conc. of amino acid	Kind of basic soln.	0.2% albumin (ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0
		Amino acid soln. (ml)	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	1.0	1.5	2.0	2.0
		Water (ml)	2.0	1.95	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.0	0.5	0	2.0
		Basic soln. (ml)	20	20	20	20	20	20	20	20	20	20	20	20	20
Arginine, 1.0%	$CoCl_2$	I (mm)	69	—	56	—	49	—	38	29	22	max	—	—	max
		II (mm)	94	—	73	—	62	—	55	41	34		—	—	
Arginine, 1.0%	luteo salt	I	37	—	37	33	25	20	16	—	—	max	—	—	max
		II	70	—	70	64	55	49	45	—	—		—	—	
Histidine, 0.1%	$CoCl_2$	I	77	—	86	88	83	—	77	—	—	83	76	72	53
		II	109.5	—	110	102	88	—	80	—	—				23
Histidine, 0.1%	luteo salt	I	64	—	63	—	—	—	63	—	—	—	64	66	29
		II	151	—	146	—	—	—	140	—	—	—	139	140	41
Histidine, 1.0%	$CoCl_2$	I	68	80	77	—	65	—	59.5	—	—	44	—	—	17
		II	100	82		—	76.5	—	88.5	—	—	104	—	—	160.5
Histidine, 1.0%	luteo salt	I	38	—	41.5	41	—	—	41	—	—	36	36	36	—
		II	101	—	97.5	96.5	—	—	93	—	—	75	69	66	—
Phenylalanine, 1.0%	$CoCl_2$	I	68	—	62	—	61	—	60	—	—	36	32	26	max
		II	100	—	85	—	77	—	74	—	—	52	44	40	
Tryptophane, 1.0%	$CoCl_2$	I	59	—	61	—	—	—	55	—	—	52	—	46	max
		II	90	—	87	—	—	—	80	—	—	71	—	63	

Change of the albumin-protein wave in the addition of the four kinds of amino acid solution are presented in Figs. 39–46 and Table 7.

The decrease of protein wave was observed when the solutions of arginine, phenylalanine and tryptophane were added but they did not changed the shape of the double protein wave to single protein wave.

Histidine decreased the wave height of protein wave and also changed the shape of the wave from double wave to single wave. The change of protein wave by the addition of histidine solution was observed merely in ammoniacal cobaltic (II) solution and not in ammoniacal cobaltic (III) solution. In ammoniacal cobaltic (III) solution all amino acids did not changed the shape of the protein wave, though they decreased the height of protein wave.

Histidine produces a single catalytic wave in ammoniacal cobaltic (II) solution, but it does not produced the catalytic wave in ammoniacal cobaltic (III) solution, so the wave produced in ammoniacal cobaltic (II) solution may be a peculiar one which is resemble to cystine wave.

It seems that the peculiar wave is similar wave which we observed in alcoholic extractives of reddy meat. The change of the protein wave by the addition of histidine solution is same as when the alcoholic extractives of reddy meat is added to proteins solution. Therefore the originals of the different wave observed in different species of aquatic animals and between in common muscle and dark colored muscle of fish mainly due to the histidine content of them.

The degree of interference of amino acid to protein wave is as following order:

Histidine>arginine and phenylalanine>tryptophane

6. A trial of the determination for meat freshness.

There are many methods to determine the freshness of meat, these are organoleptic, biological, physical and chemical test.

Organoleptic test is the most convenient and accurate method, but it is not scientific method. In biological test, it is decided for the first stage of putrefaction of meat by number of bacteria of 10^5–10^8/g. Physical test such as by the elasticity of muscle and by the electric resistance of meat is not in practical use because the results are not uniform in the species. In chemical test most of the methods are examined the substance produced during the putrefaction of meat such as volatile-basic nitrogen, amino-nitrogen, hydrogen sulfide, indol, etc. Others are by the measurement of pH, reducing pigment and determination of fluorescence. Above all, nowadays, creditable methods are the measurement of volatile-basic nitrogen[47–51] and pH value[52–53]. A method, we are now present, may not be suit for practical use of meat freshness immediately.

It is already known that SH groups are releaved from protein [54,55] molecule during the denaturation and the contents are used for the measurement of freshness of meat by ANSON and MIRSKY[56–61].

We examined the increase and decrease of protein wave for meat extractives during the putrefaction of meat, expecting the protein wave change in height of wave with the freshness of meat.

Fresh meat of carp and cuttle fish were elapsed at a room temperature and

observed the increase and decrease of protein wave. The measurement of pH, contents of volatile-basic nitrogen and organoleptic test were also tested as the confer examination.

In polarography a method which was polarographed by the addition of water extractives and ammoniacal cobaltic (II) solution, and method by filtrate reaction technique were used. The preparation of filtrate reaction technique was that, water extractives of meat added with 1 N KOH solution for exactly 30 minutes

Fig. 47. Change of protein wave with the meat freshness—Cuttle fish meat.
A–D, 2.0 ml of water extractives of meat (extracted 50 times)+20 ml of ammoniacal coboltic (II) solution; E–H, filtrate reaction technique. A, E, initial; B, F, elapsed one day; C, G, 2 days; D, H, 4 days. Sensitivity, 0.100 μA/mm.

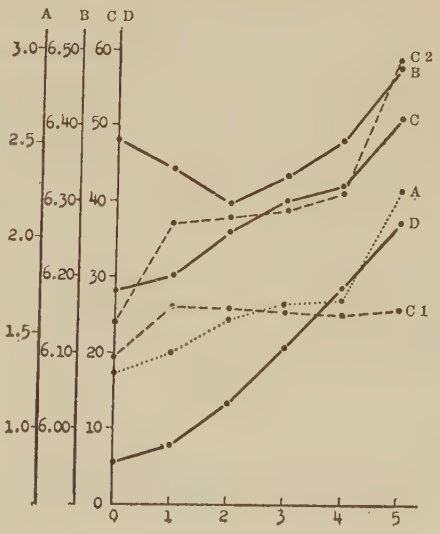


Fig. 48. Change of protein wave, pH and contents of volatile-basic nitrogen.—cuttle fish meat.

Change of protein wave, pH and contents of volatile basic nitrogen during the putrefaction of cuttle fish meat.

A, the ratio of the first wave and the second wave in filtrate reaction technique; B, pH value; C, height of protein wave in ammoniacal cobaltic (II) solution; C1, C2, height of the first and second wave in filtrate reaction technique; D, contents of volatile-basic nitrogen (mg %). Abscissa, numbers of day elapsed.

then added of 20% sulfosalicylic acid and stood exactly 10 minutes filtrate was polarographed in addition of basic solution (ammoniacal cobaltic (III) solution for reaction technique).

The results for meat of cuttle fish tested for 5 days are presented in Figs. 47, 48 and Table 8. The first stage of putrefaction of cuttle fish meat decided

Table 8. Change of protein wave during the putrefaction of cuttle fish meat.
Protein wave A, protein wave of water extractives of meat in ammoniacal cabaltic (II) solution; protein wave B, protein wave of water extractives of meat by the filtrate relation technique., I, the first wave; II, the second wave of protein waves.

Days elapsed	0	1	2	3	4	5
pH	6.38	6.34	6.30	6.33	6.38	6.48
Volatile basic N (mg%)	5.2	7.6	13.1	20.5	28.5	37.2
Protein wave A (mm)	28.0	30.0	36.2	40.0	42.0	51.0
Protein wave B I (mm)	19.5	26.0	25.8	25.5	25.0	26.0
Protein wave B II (mm)	24.0	37.0	37.8	39.0	42.0	59.0

four and five days after leaving, by measurement of pH value and contents of volatile-basic nitrogen. In this stage protein waves were not so clear in the change of height though they increase gradually with the putrefaction, but the protein wave produced by the filtrate reaction technique showed a cleary change that the wave height is remarkably increased in this stage. It is almost concided that the first stage of putrefaction of meat by the measurement of chemical test and polarographic test.

For carp meat the same results were obtained as presented in Figs. 49–51 and Table 9.

It is considered that the increasing height of protein wave due to the increasing of SH and proteinous compounds.

It is somewhat difficult to measure the first stage of putrefaction by the wave height of protein which is produced in water extractives and ammoniacal cobaltic (II) solution, because the change of the wave height is not so distinct during the putrefaction, but it is possible to determine the freshness of meat by measuring of the height of protein wave in the sensitivity of μA/mm, as decided the first stage of putrefaction by the content of volatile-basic nitrogen in 30 mg%.

It is avilable to determine the freshness by the height of protein wave produced in the filtrate reaction technique, especially by a ratio of the first and second of the protein wave, because the ratio is remarkably changed in the first stage of putrefaction.

7. Denaturation of protein by γ-ray irradiation

The authors have been studied the influence of γ-ray irradiation for aquatic animals and the sterilization of food by γ-ray irradiation[65–75].

In these studies it is necessary to study the denaturation of protein in

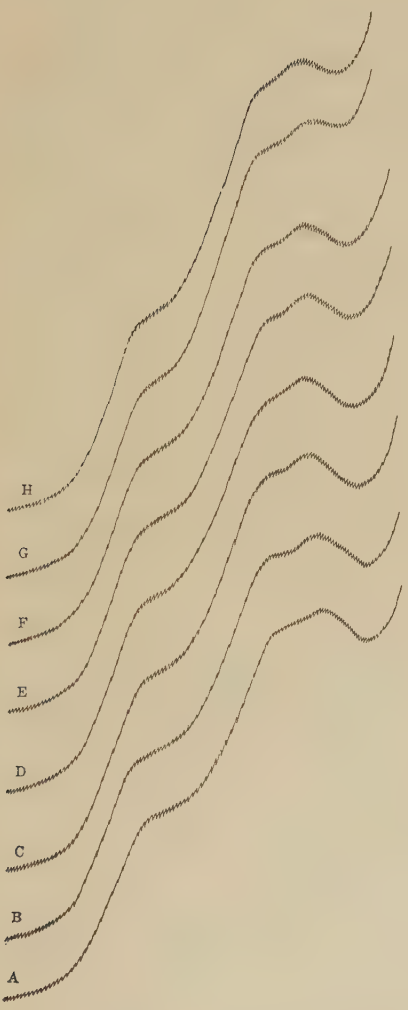


Fig. 49. Change of protein wave with meat freshness—Carp meat.

1.0 ml. of water extractives of meat (extracted 10 times)+20 ml. of ammoniacal cobaltic (II) solution. A, initial; B, elapsed one day; C, 2 days; D, 3 days, E, 4 days; F, 5 days; G, 7 days; H, 8 days. Sensitivity, 0.080 μA/mm.

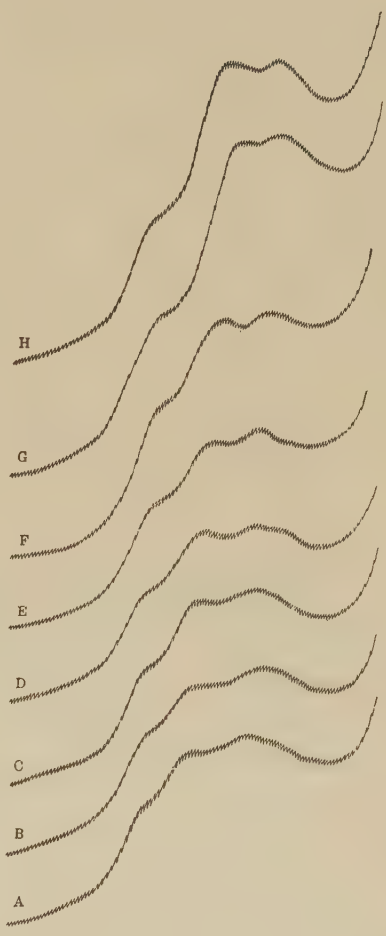


Fig. 50. Change of protein wave with meat freshness—Carp meat.

Filtrate reaction technique. A, initial; B, elapsed one day; C, 2 days; D, 3 days, E, 4 days; F, 5 days; G, 7 days; H, 8 days. Sensitivity, 0.060 μA/mm.

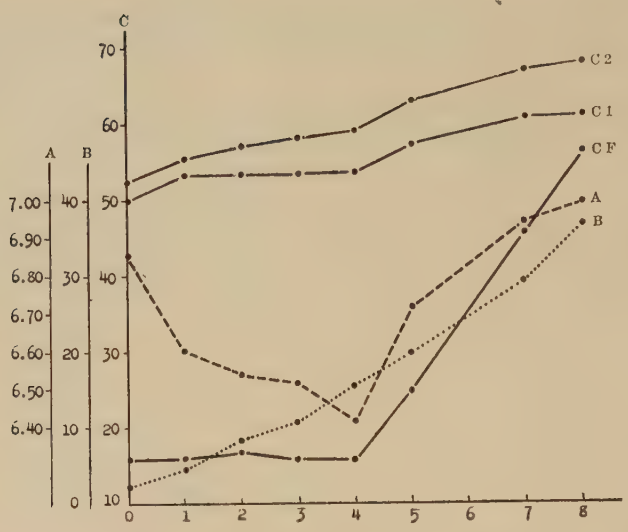


Fig. 51. Change of protein wave, pH and volatile basic nitrogen during the putrefaction of carp meat.

A, pH value; B, contents of volatile basic nitrogen (mg %); C, height of protein wave (mm); Cl, C2, height of protein wave in ammoniacal cobaltic (II) solution (mm); CF, filtrate reaction technique. Abscissa, numbers of day elapsed.

Table 9. Change of protein wave during the putrefaction of carp meat.

$HgCl_2$ reaction, *cf.* literature 62-64, protein wave A, protein wave of water extractives of meat in ammoniacal cobaltic (II) solution; protein wave B, protein wave of water extractives of meat by the filtrate reaction technique.

Days elapsed	0	1	2	3	4	5	7	8
pH	6.85	6.60	6.52	6.51	6.41	6.71	6.92	7.00
Volatile basic N (mg%)	2.3	4.6	8.3	11.0	16.0	20.0	29.5	37.0
$HgCl_2$ reaction I	—	—	+±	±+	++	++	++	##
〃 II	—	—	−−	−−	±±	−+	++	##
Protein wave A (mm)	52.5	53.5	57.0	58.0	59.0	63.0	67.0	68.0
〃 B (mm)	16.0	16.0	17.0	16.0	16.0	25.0	46.0	57.0

a living body and foods in biologically and dietetically.

A neccessary γ-ray dosage to sterilization of food[76-87] is about 2×10^6 roentgens[79,81-86], and the irradiated foods are preserved well for two or four weeks. But some of them decrease their nutritive value and have undesirable odor during the γ-ray irradiation.

Therefore, many problems on this point are remained for the sterilization of food. The study on denaturation of protein is one of the subject which should to give a solution immediately. The studies on the denaturation of protein are considerable and especially polarographic study is more rare.

Therefore, polarographic studies on the denaturation of several proteins are examined. The following subjects are studied by polarographic protein wave:

influence of γ-ray irradiation for 1) cuttle serum, 2) albumin solution, 3) globulin solution and 4) fish meat.

1) Influence of γ-ray irradiation for cuttle serum.

As materials for this experiment cuttle serum was prepared immadiately after slaughter. The serum and serum diluted with 50 times of water were irradiated with γ-ray from Co^{60} (100 curie) in glass ampoule, in the dosage of 58,6000, 110,400, and 322,000 roentgens respectively. Control serum was leaved in the same condition except from irradiation.

These serum was tested by polarographic, organoleptic and spectrophotometric method. The polarographic test was examined in two ways, the one polarographed the serum in ammoniacal cobaltic (II) solution and the other polarographed by the filtration reaction technique. After irradiation the undiluted serum was diluted 50 times of water and the diluted serum was tested in that condition. The electrolytic solution for filtrate reaction technique was prepared that, 2.0 ml of diluted serum added with 1.0 ml of 1 N KOH solution stood for exactly 30 minutes then added 2.0 ml of 20% sulfosalicylic acid solution and leaved exactly for 10 minutes then filtered, the filtrate was polarographed in ammoniacal cobaltic (III) solution for filtrate reaction technique.

Change of the color, odor and pH value of serum after irradiation are presented in Table 10. Irradiated serum have a tinge of black with increasing dosage

Table 10. Change of color of irradiated serum.

Irradiated dosage	Initial	Control	58,600 r	110,400 r	322,000 r
Color of undil. serum after irradiation	dark red	dark red	dark red	blackish red	blackish red
Do, dil. 50 times of water	bright red	bright red	bright red	brownish red	blackish red
Do, pH	6.4	6.4	6.8	6.4	6.2
Odor	bloody odor	bloody red	slightly ← irradiated odor → strong		
Color of dil. serum after irradiation					
Color	bright red	bright red	brownish red	reddish brown	grayish brown
pH	6.4	6.4	6.8	6.4	5.8
Odor	bloody odor	bloody odor	slightly ← irradiated odor → strong		

of γ-ray and it is distinct in diluted serum. As shown in Fig. 52, absorbancy of serum in wave length of 500–600 mμ decreases after the irradiation. This indicates the decomposition of serum pigment by γ-ray irradiation. After irradiation the odor of the serum have a peculiar odor regard as irradiation odor, pH values are not so changed in the irradiation dosage of less than 3×10^5 r.

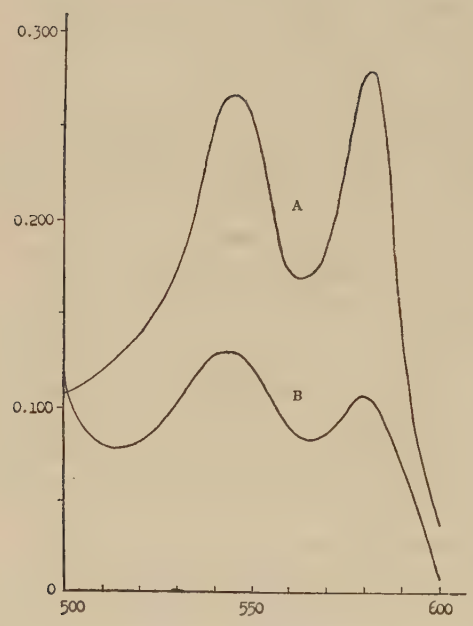


Fig. 52. Absorbancy of irradiated serum.
A, control, unirradiated; B, irradiated 3.2×10^5 r.; abscissa, wave length (mμ); ordinarly, optical density.

Protein wave which is obtained to the serum in ammoniacal cobaltic (II) solution increases the height of wave in the dosage of 5.8×10^4 r and then decreases the wave height with the further increasing dosage (Fig. 53).

The increase and decrease of protein wave are same as reported by WENIG[88,89] in the irradiation of ultraviolet ray to protein solution.

In the irradiation to undilted serum the increase and decrease of protein wave is not so distinct as presented in Fig. 54. The change of protein wave observed obviously by filtrate reaction technique as presented in Fig. 55. Even the filtrate reaction technique irradiated to undiluted serum is not so change in height of wave, therefore it is apparent that undiluted serum does not suffer less denaturation than diluted serum during the γ-ray irradiation. (Fig. 56).

Fig. 53. Polarogram of irradiated diluted serum.
2.0 ml of dil. serum+20 ml. of ammoniacal cobaltic (II) solution. A, control; B, irradiated 58,600 r; C, 110,000 r; D, 322,000 r. Sensitivity, 0.100 μA/mm.

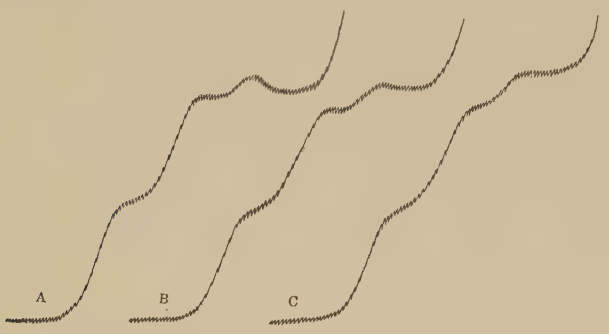


Fig. 54. Polarogram of irradiated serum.
2.0 ml. of dil. serum 50 times of water after irradiation+20 ml. of ammoniacal cobaltic (II) solution. A, control; B, irradiated 58,600 r; C, 322,000 r. Sensitivity, 0.100 μA/mm.

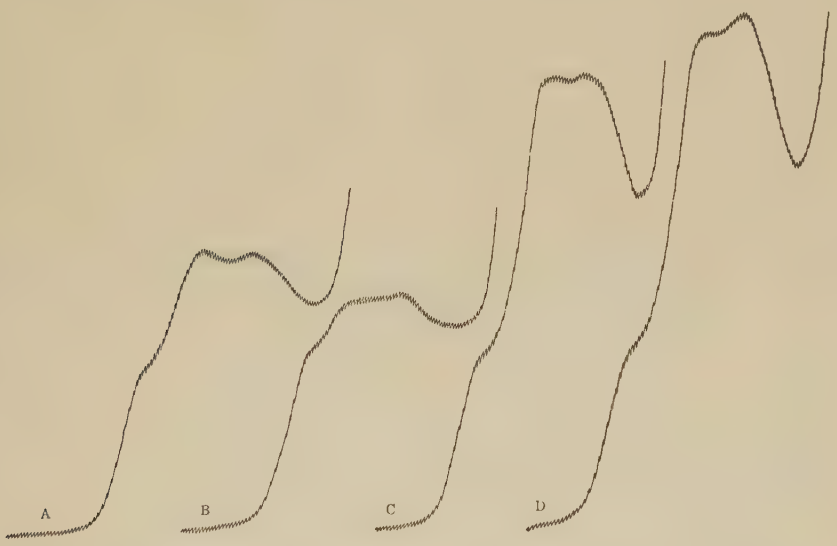


Fig. 55. Polarogram of irradiated dilute serum (filtrate reaction technique).
A, initial; B, control; C, irradiated 58,600 r; D, 322,000 r. Sensitivity, 0.060 μA/mm.

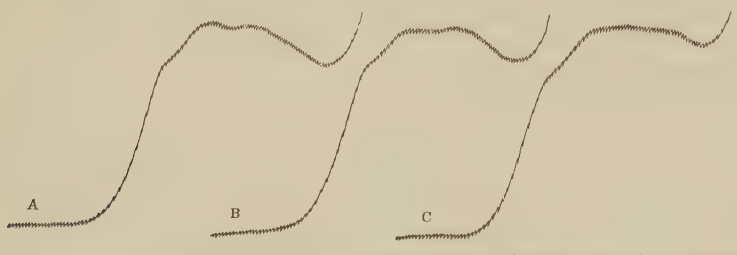


Fig. 56. Polarogram of irradiated undiluted serum (filtrate reaction technique).
A, control; B, irradiated 58,600 r; C, 322,000 r. Sensitivity, 0.060 μA/mm.

2) Influence of γ-ray irradiation to albumin solution[90-93].

Albumin solutions in the concentration of 0.2, 0.5 and 1.0% were irradiated to γ-ray and the changes of the characteristics of them were observed during the irradiation. Polarographic observations were done in the change of protein wave in ammoniacal cobaltic (II) or (III) solution and filtrate reaction technique. Particularly spectrophotometric and organoleptic test and measurement of pH value were done as a confer examination.

Color, odor and pH value of 0.2, 0.5 and 1.0% albumine solution which irradiated in the range of $6.4\text{–}79.4\times10^4$ r. is presented in Table 11.

Table 11. Change of color and pH value of irradiated albumin solution. +, irradiated odor; * pH value of electrolytic solution in the addition of albumin and ammoniacal cobaltic (II) solution.

Concentration	0.2% soln.				0.5% soln.				1.0% soln.			
Irradiation dosage (r)	color	odor	pH	pH*	color	odor	pH	pH*	color	odor	pH	pH*
Initial	no	no	6.20	9.50	no	no	6.22	9.50	no	no	6.30	9.50
Control	no	no	6.12	9.50	no	no	6.22	9.48	no	no	6.25	9.47
64,000	no	no	6.11	9.47	no	no	6.28	9.45	no	no	6.33	9.46
96,000	no	no	6.10	—	no	no	6.30	—	no	no	6.35	—
192,000	no	+	6.19	9.42	no	+	6.34	9.50	no	+	6.41	9.40
320,000	slightly yellow	+	6.20	—	slightly yellow	+	6.50	—	slightly yellow	+	6.49	—
384,000	〃	+	6.26	9.48	〃	+	6.50	9.48	〃	+	6.55	9.47
441,600	〃	+	6.22	—	〃	+	6.49	—	〃	+	6.52	—
585,600	〃	+	6.18	9.40	〃	+	6.45	9.51	〃	+	6.60	9.45
796,800	〃	+	6.11	9.40	〃	+	6.49	9.35	〃	+	6.60	9.40

In the irradiation dosage more than 3×10^5 r. the albumin solution have a tinge of slightly yellow and emitts irradiation odor. The odor and color are distinct according with the increase of irradiation dosage.

The pH value is not so changed during the irradiation that the concentration of albumin changes from 0.2 to 1.0%. Protein waves of the albumin solution after irradiation are shown in Figs. 57–59.

The decrease of the protein wave is observed in each concentration of albumin and the decrease of wave height is plainly appeared in low concentration of albumin solution. The most change in each solution occurs in irradiation dose of 8×10^6 r. It is sure that albumin solution suffers a severe deterioration in irradiation of 8×10^6 r.

Absorbancy to albumin solution with several dosage is shown in Figs. 60–62. Increase of the absorbancy in wave length of 280 mμ was observed after

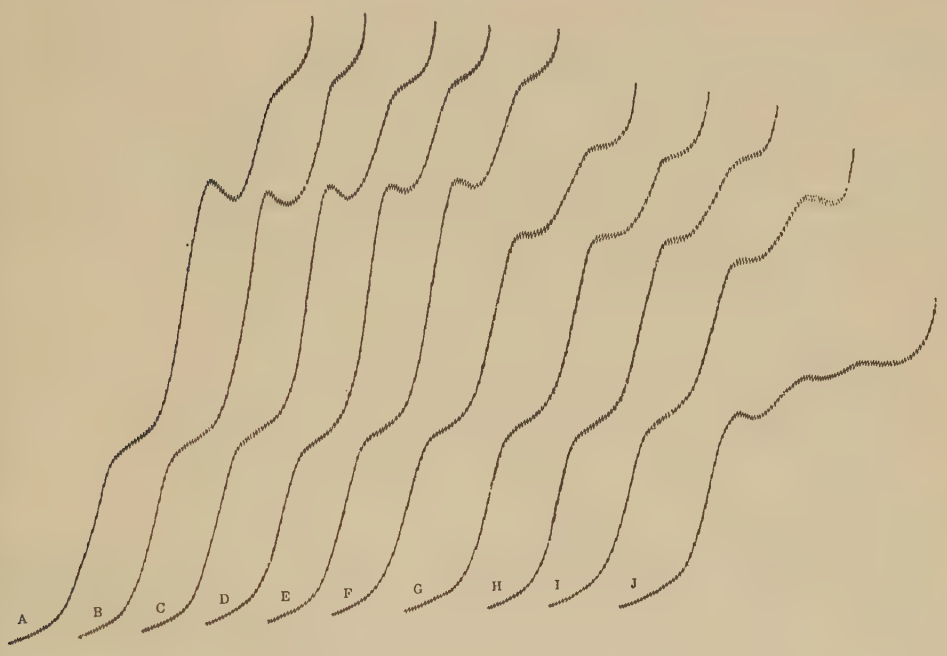


Fig. 57. Polarograms of irradiated albumin solution (0.2%).
2.0 ml of 0.2% albumin solution+20 ml of ammoniacal cobaltic (II) solution. A, initial; B, control; C, irradiated 6.4×10^4 r; D, 9.6×10^4 r; E, 19.2×10^4 r; F, 32×10^4 r; G, 38.4×10^4 r; H, 44.2×10^4 r; I, 58.6×10^4 r; J, 79.7×10^4 r. Sensitivity, 0.100 μA/mm.

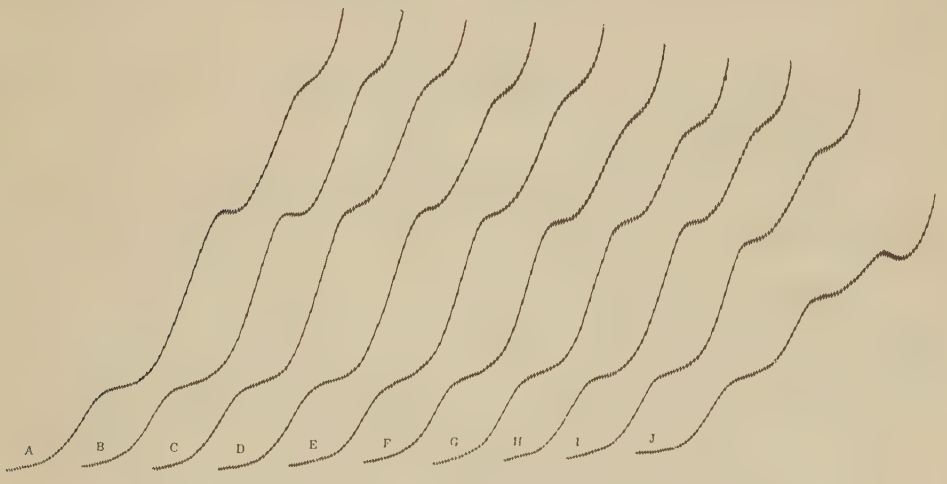


Fig. 58. Polarograms of irradiated albumin solution (0.5%).
2.0 ml of 0.5% albumin solution+20 ml of ammoniacal cobaltic (II) solution. A, initial; B, control, C, irradiated 6.4×10^4 r; D, 9.6×10^4 r; E, 19.2×10^4 r; F, 32×10^4 r; G, 38.4×10^4 r; H, 44.2×10^4 r; I, 58.6×10^4 r; J, 79.7×10^4 r. Sensitivity, 0.200 μA/mm.

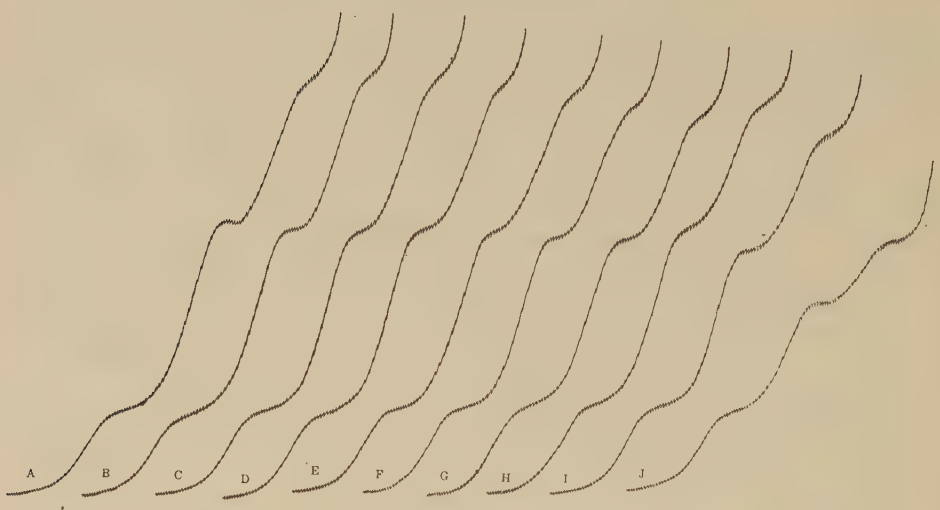


Fig. 59. Polarograms of irradiated albumin solution (1.0%).
1.0 ml of 1.0% of albumin solution+20 ml of ammoniacal cobaltic (II) solution. A, initial; B, control, C, irradiated 6.4×10^4 r; D, 9.6×10^4 r; E, 19.2×10^4 r, F, 32×10^4 r; G, 38.4×10^4 r; H, 44.2×10^4 r; I, 58.6×10^4 r; J, 79.7×10^4 r. Sensitivity, 0.200 μA/mm.

the irradiation. Clearly increase of absorbancy occurs in 0.2% solution irradiated with 32×10^4 r., 0.5% solution irradiated with 58.6×10^4 r. and 1.0% solution irradiated with 79.7×10^4 r. respectively.

The increase of the absorbancy in wave length of 280 mμ was already found in the denaturation by the irradiation of ultraviolet ray and α-ray[94,95] and it was recognized to ulternate from tyrosine and phenylalanine to dihydroxy phenyl alanine.

The change in protein wave height occurs in the same irradiation dosage as in absorbancy of albumin, though the one decreases the wave height and the other increases the absorbancy.

Therefore it is sure that albumin suffers a serious deteriolation by γ-ray irradiation in low concentration of solution. At the same time it is clarified that the polarographic protein wave is available to investigate the denaturation of protein.

As reported by WENIG[88,89] in the irradiation of ultraviolet ray protein wave increased in a certain dosage of irradiation and then the wave decreased with the increase of irradiation dosage, but we could not observed the increase of protein wave during the irradiation of γ-ray as above mentioned, so we examined the change of the wave in the lower irradiation dosage less than 3×10^5 r.

Here the filtrate reaction technique was also used to examine the details of the change of protein during the irradiation.

The results are presented in Figs. 63–68. In all cases the protein wave decreased little in height but did not increased during the irradiation. HEEREN et al[90] reported the change of protein wave during the irradiation of X-ray less

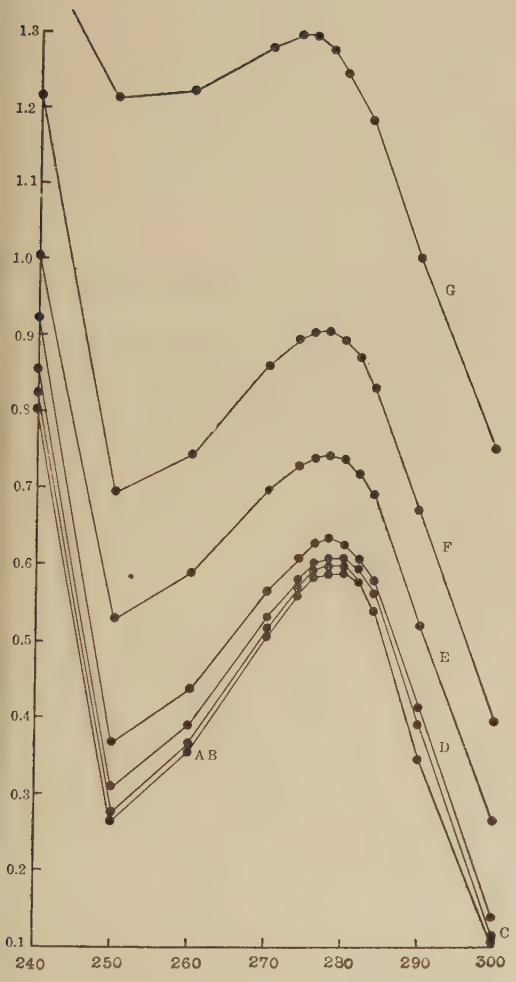


Fig. 60. Absorbancy of irradiated albumin solution (0.2%).

Measured the solution diluted two times of water. Abscissa, wave length (mμ); Ordinary, optical density. A, initial; B, control; C, irradiated 6.4×10^4 r; D, 9.6×10^4 r; E, 32×10^4 r; F, 58.6×10^4 r; G, 79.7×10^4 r.

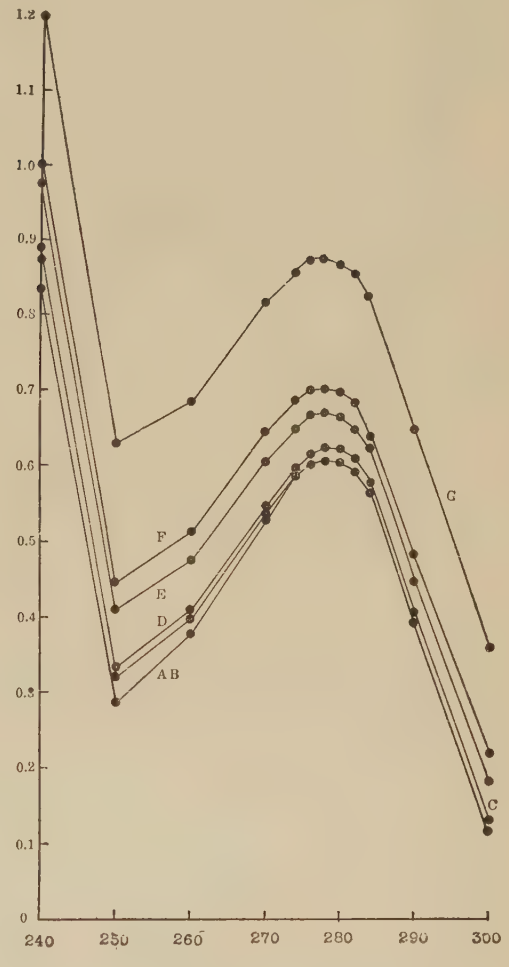


Fig. 61. Absorbancy of irradiated albumin solution (0.5%).

Measured the solution diluted five times of water. Abscissa, wave length (mμ); ordinary, optical density. A, initial; B, control; C, irradiated 6.4×10^4 r; D, 9.6×10^4 r; E, 32×10^4 r; F, 58.6×10^4 r; G, 79.7×10^4 r.

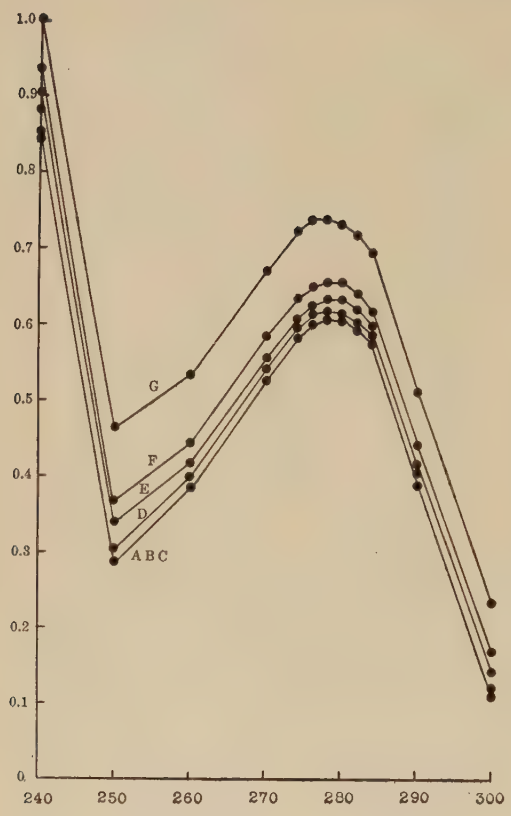


Fig. 62. Absorbancy of irradiated albumin solution (1.0%).

Measured the solution diluted 10 times of water. Abscissa, wave length (mμ) ordinary, optical density. A, initial; B; control; C, irradiated $6.4\times10_4$ r; D, 9.6×10^4 r; E, 32×10^4 r F, 58.6×10^4 r; G, 79.7×10^4 r.

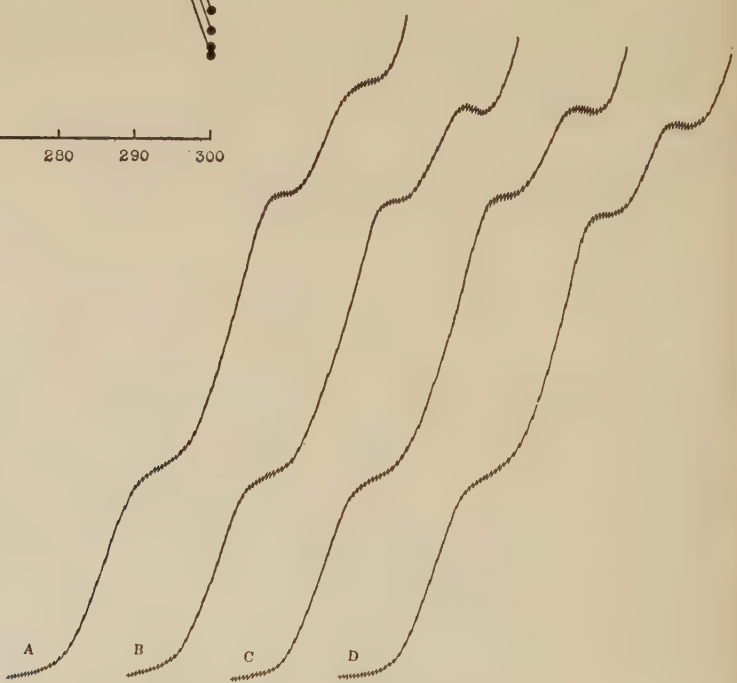


Fig. 63. Polarograms of irradiated albumin solution (0.2%).

2.0 ml of irradiated albumin solution+20 ml of ammoniacal cobaltic (II) solution. A, control; B, irradiated 1×10^4 r; C, 3×10^4 r; D, 5×10^4 r. Sensitivity, 0.100 μA/mm.

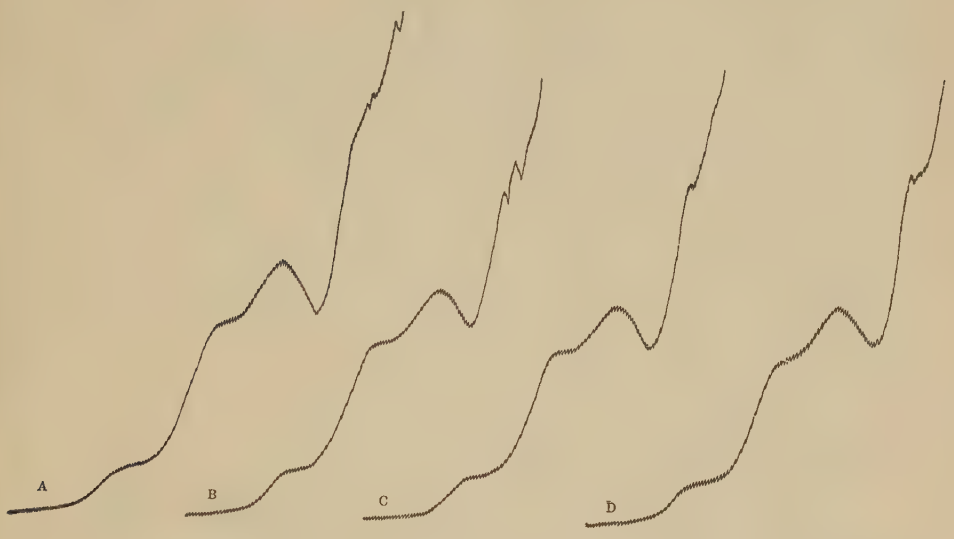


Fig. 64. Polarograms of irradiated albumin solution (0.2%)—filtrate reaction technique.
A, control; B, irradiated 1×10^4 r; C, 3×10^4 r; D, 5×10^4 r. Sensitivity, 0.200 μA/mm.

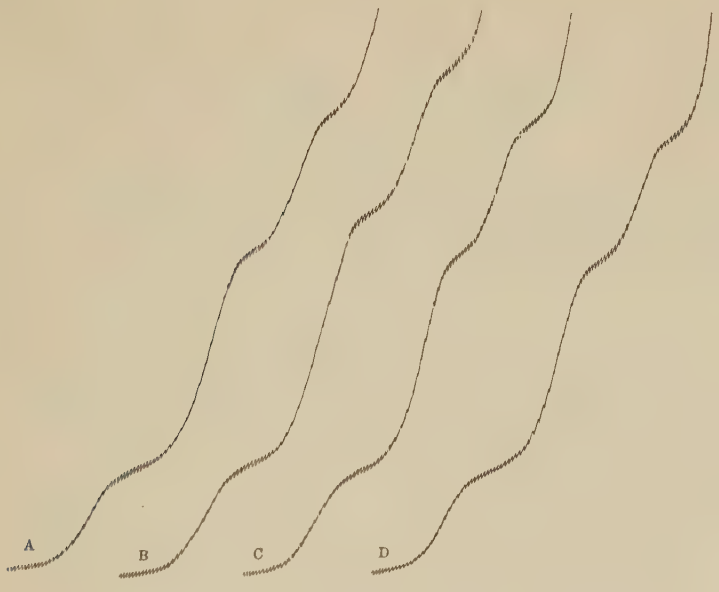


Fig. 65. Polarograms of irradiated albumin solution (0.5%).
A, control; B, irradiated 1×10^4 r; C, 3×10^4 r; D, 5×10^4 r. Sensitivity 0.200 μA/mm.

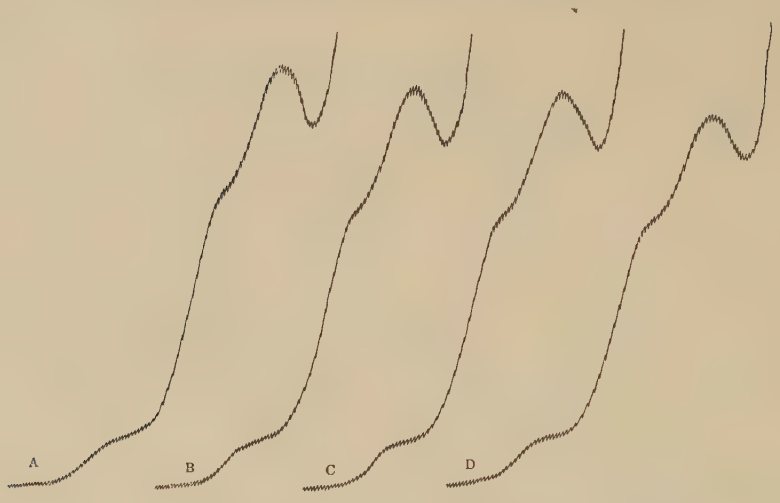


Fig. 66. Polarograms of irradiated albumin (0.5%) — filtrate reaction technique. A, control; B, irradiated 1×10^4 r; 3×10^4 r; D, 5×10^4 r. Sensitivity, 0.400 μA/mm.

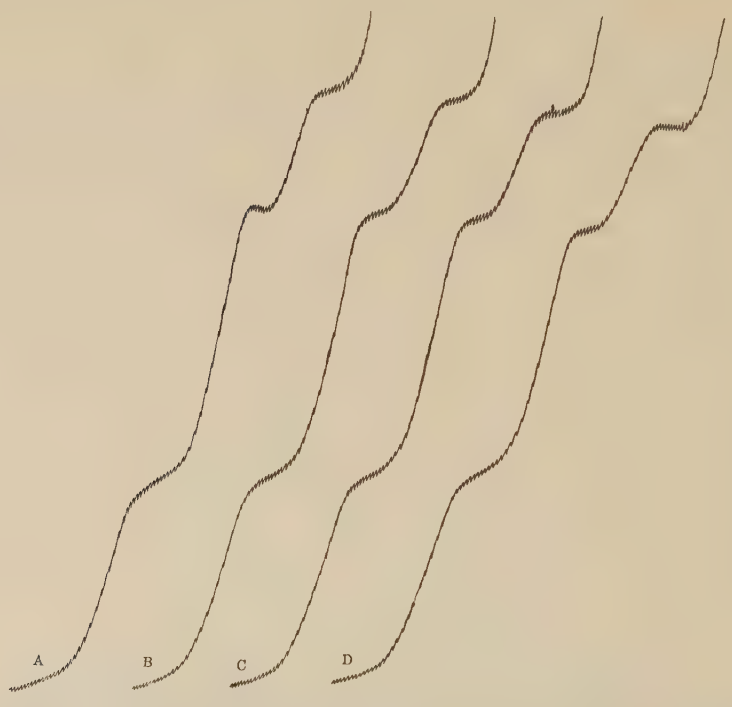


Fig. 67. Polarograms of irradiated albumin solution (0.2%). A, control; B, irradiated 1×10^5 r; C, 2×10^5 r; D, 3×10^5 r. Sensitivity, 0.100 μA/mm.

Fig. 68. Polarograms of irradiated albumin solution (0.2%)—filtrate reaction technique. A, control; B, irradiated 1×10^5 r; C, 2×10^5 r; D, 3×10^5 r. Sensitivity, 0.200 μA/mm.

than 1×10^5 r, and obtained unchanged protein wave compared with control. Accordingly, in the irradiation less than 3×10^5 r the denaturation of albumin is not so distinct as in the further irradiation.

3) Influence of the γ-ray irradiation to globulin.

Globulin solutions of 0.1% and 0.4% were irradiated as the experiment of albumin, and observed the protein wave. As shown in Figs. 69–71 the protein wave increased in the irradiation dose of 3×10^5 r, then the protein wave decreased with increase of irradiation doses. The increase and decrease of protein wave was same as irradiated for ultraviolet ray[92].

In this studies the arrangement of γ-ray irradiation containing 1 kilocuries of cobalt-60 was just completed in our laboratory, and it was possible to irradiate comparative many materials at the same time, though it was not enough to study the sterilization of food. The highest irradiation dose of this arrangement is 2.4×10^5 r/hour.

We examined the denaturation of albumin and globulin irradiated by γ-ray. by this arrangement. The 0.2% and 0.5% solution of albumin and globulin were prepared and irradiated with γ-ray in glass ampoule in dose range of 3.5×10^5–1×10^7 r.

The state and pH value of each solution after the irradiation are presented in Table 12. The pH values somewhat change in acidic side after the irradiation. The precipitates of albumin solution are not formed even in the irradiation

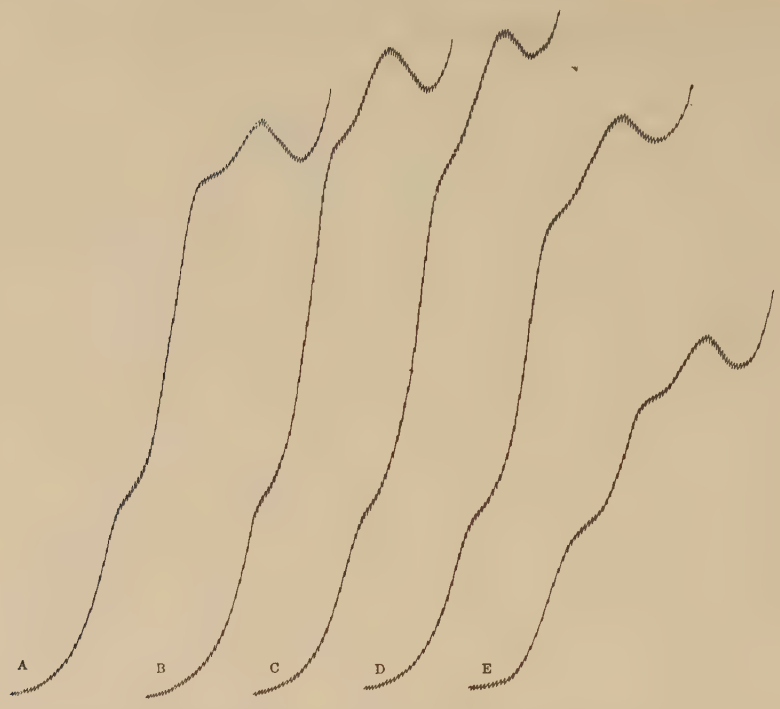


Fig. 69. Polarograms of irradiated globulin solution (0.1%).
3.0 ml of 0.1% globulin solution+20 ml of ammoniacal cobaltic (II) solution.
A, control; B, irradiated 9.6×10^4 r; C, 21×10^4 r; D, 32×10^4 r; E, 5×10^5 r.
Sensitivity, 0.060 μA/mm.

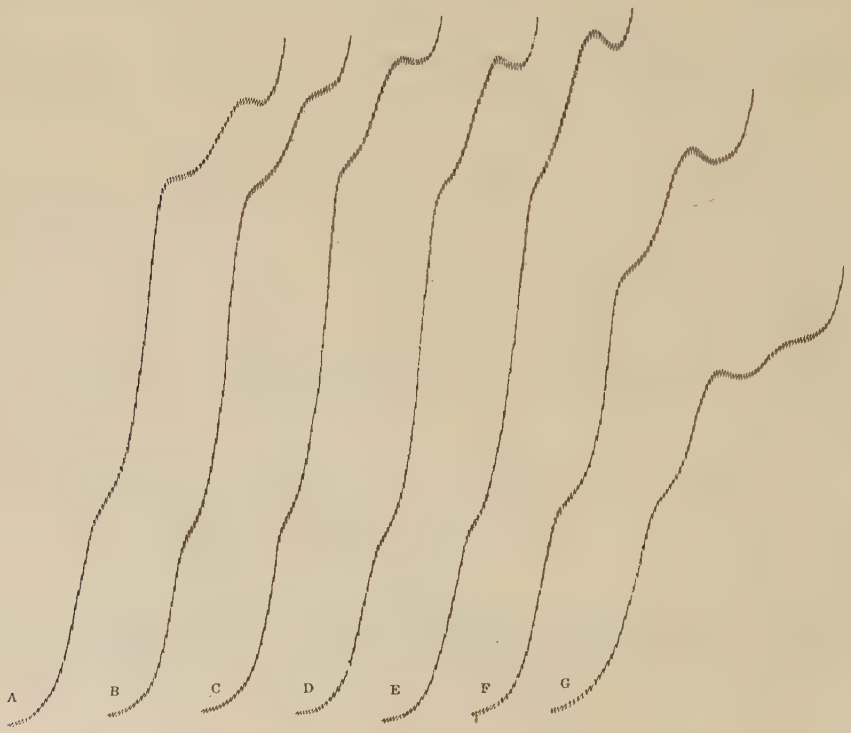


Fig. 70. Polarograms of irradiated of globulin solution (0.4%).
1.0 ml of 0.4% globulin solution+20 ml of ammoniacal cobaltic (II) solution.
A, control; B, irradiated 5×10^4 r; C, 1×10^5 r; D, 2×10^5 r; E, 3×10^5 r; F, 4×10^5 r; G, 7.4×10^5 r.

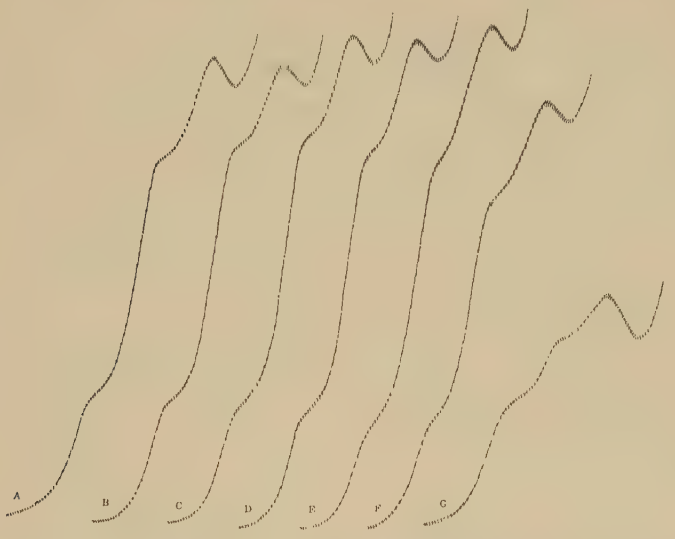


Fig. 71. Polarograms of irradiated globulin solution (0.4%).
1.0 ml of 0.4% globulin solution+20 ml of ammoniacal cobaltic (III) solution. A, control; B, irradiated 5×10^4 r; C, 1×10^5 r; D, 2×10^5 r; E, 3×10^5 r; F, 4×10^5 r; G, 7.4×10^5 r.

Table 12. Change of state and pH value of irradiated albumin and globulin. Trans., transparency; mudd., muddiness; p.p.t., precipitate; coag., coagulation.

Irradiated dosage ($\times10^4$r)	Albumin				Globulin			
	0.2%		0.5%		0.2%		0.5%	
	state	pH	state	pH	state	pH	state	pH
0	opacity	6.55	opacity	6.60	trans.	5.95	trans.	6.20
35	opacity	6.50	opacity	6.72	trans.	5.77	trans.	6.02
60	trans.	6.52	trans.	6.55	mudd.	5.72	mudd.	5.92
120	trans.	6.48	trans.	6.52	p.p.t.	5.68	p.p.t.	5.87
400	trans.	6.45	trans.	6.50	coag.	5.60	coag.	5.87
1000	trans.	6.40	trans.	6.42	coag.	5.56	coag.	5.60

dose of 1×10^7 r, but globulin solutions form a precipitate in the irradiation dose of 12×10^5 r and coagulated in 4×10^6 r.

Polarograms of albumin and globulin after the irradiation are shown in Figs. 72–75. In the irradiation dose of more than 6×10^5 r albumin and globulin decrease their height of protein wave, especially it is more distinct in globulin than in albumin. The decrease of waves of globulin in the irradiation of more than 1.2×10^6 r is surely caused by the precipitation of globulin but the decrease of the wave in albumin and globulin irradiated with less than 6×10^5 r assumed to cause of the molecular change of proteins.

Fig. 72. Polarograms of irradiated albumin (0.2%).

2.0 ml of 0.2% albumin+20 ml of ammoniacal cobaltic (II) solution. A, control; B, irradiated 3.5×10^5 r; C, 6×10^5 r; D, 12×10^5 r; E, 4×10^6 r; F, 1×10^7 r. Sensitivity, 0.100 μA/mm.

Fig. 73. Polarograms of irradiated albumin (0.5%).

1.0 ml of 0.5% albumin+20 ml of ammoniacal cobaltic (II) solution. A, control; B, irradiated 3.5×10^5 r; C, 6×10^5 r, D, 12×10^5 r; E, 4×10^6 r; F, 1×10^7 r. Sensitivity, 0.200 μA/mm.

McARDLE et al[98] studied the denaturation of albumin for γ-ray irradiation and clarified the increase of viscosity and SH content and unchanged amino nitrogen in the dose level of 1.5×10^6 r. They demonstrated that the molecules of alubumin were transformed from a globular form to an asymmetrical molecule

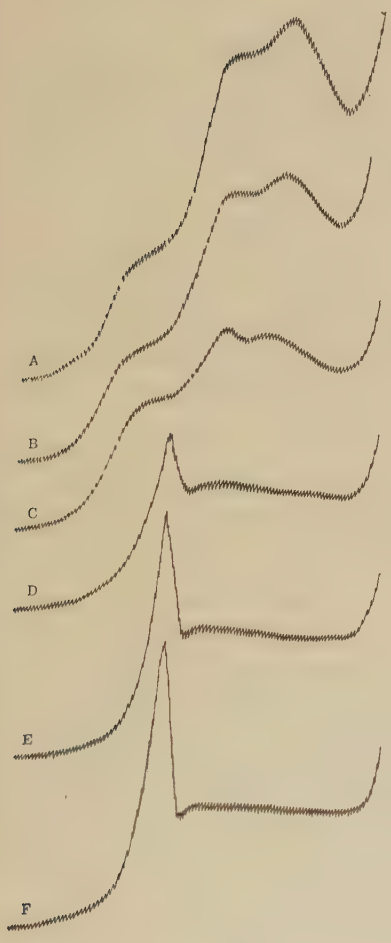


Fig. 74. Polarograms of irradiated globulin (0.2%).

2.0 ml of 0.2% globulin solution+20 ml of ammoniacal cobaltic (II) solution A, control; B, irradiated 3.5×10^5; C, 6×10^5 r; D, 12×10^5 r; E, 4×10^6 r; F, 1×10^7 r. Sensitivity, 0.100 μA/mm.

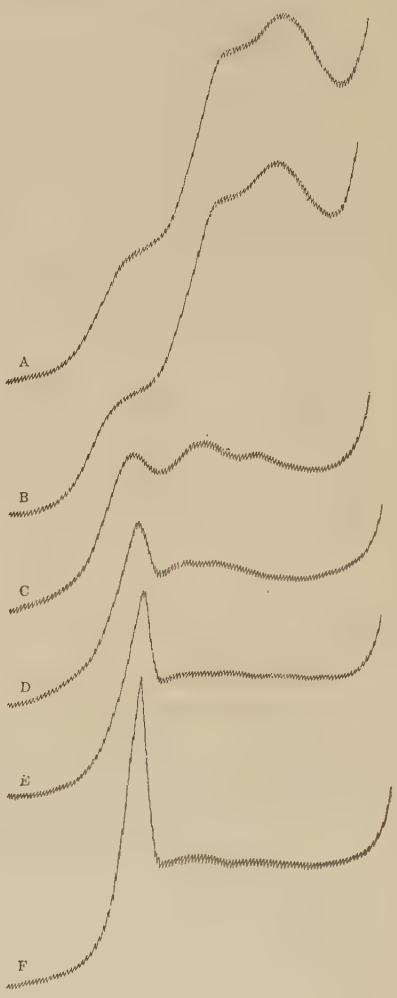


Fig. 75. Polarograms of irradiated globulin (0.5%).

1.0 ml of 0.5% globulin solution 20 ml of ammoniacal cobaltic (II) solution A, control; B, 3.5×10^5 r; C, 6×10^5 r; D, 12×10^5 r; E, 4×10^6 r; F, 1×10^7 r. Sensitivity, 0.200 μA/mm.

in the irradiation and associated the molecules when irradiation was prolonged.

In the polarographic examination the same molecular changes were concluded.

Increase and decrease of protein wave during the denaturation for heat and ultraviolet ray[99] are considered that the protein wave increases in the first stage of denaturation by the liberation of active SH groups in the molecule and

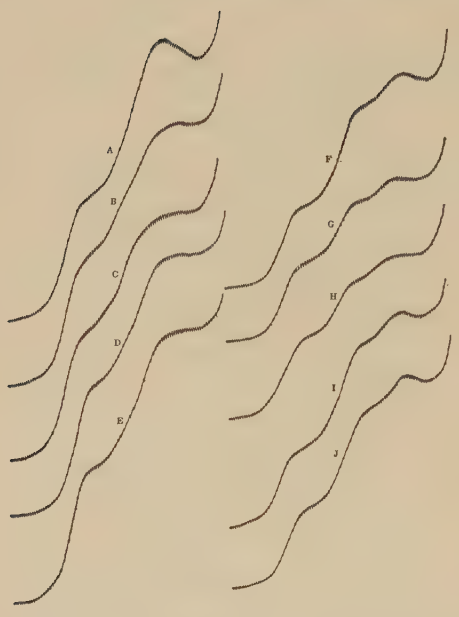


Fig. 76. Polarograms of irradiated fish meat—*Cyprinus caprio*.

A, F, water extractives of meat (extracted 10 times); B, G, irradiated; A, F, 1.3×10^6 r; C, H, irradiated; A, F, 2×10^6 r; D, I, water extractives of irradiated meat, 1.3×10^6 r; E, J, water extractives of irradiated meat, 2×10^6 r. A–E, ammoniacal cobaltic (II) solution Sensitivity, 0.100 μA/mm; F–J, ammoniacal cobaltic (III) solution. Sensitivity, 0.200μA/mm.

decrease in the second stage by the association of the active molecules to globular form.

It seems that the denaturation of protein for γ-ray irradiation does occurs in same mechanism as irradiate for ultraviolet ray.

4) Influence of the γ-ray irradiation to fish meat.

Here, we examined the alternation of protein in the irradiation of fish meat in the dose range of 1.5–2.0×10^6 r, since this level of irradiation would be required for complete sterilization.

Fresh meat of *Cyprinus caprio*, *Katsuwonus pelamis*, *Thunnus thynnus*, and *Paralichthys clivaeus* were irradiated in dose range of 5×10^5–4×10^6 r. Four kinds of meat were irradiated in double polyethylen bags and water extractives of them were irradiated in glass ampoules. The denaturation of protein contained in fish meat was examined by protein wave. These results are shown in Figs. 76–79 and Table 13. Water extractives of fish meat decrease the height of protein wave by the γ-ray irradiation but fish meat irradiated in that state does not change the protein wave.

This indicate that the proteins which is dissolved in a large quantity of water suffer more deteriolation than in low moisture state.

The denaturation of fish protein is obvious in white meat than in reddy meat.

The slightly alternation of protein in fish muscle indicates the existence of a certain substance which is protected to the denaturation of protein in meat. It may be possible to suppose the existence of protective substance because vitamin C[98] and other protective agent[100–106] suspect against the alternation of protein.

Fig. 77. Polarograms of irradiated fish meat—*Katsuwonus pelamis*.
A, F, water extractives of meat (extracted 10 times); B, G, irradiated A, F, $13.\times10^6$ r; C, H, irradiated A, F, 2×10^6 r; D, I, water extractives of irradiated meat, 1.3×10^6 r; E, J, water extractives of irradiated meat, 2×10^6 r. A–E, ammoniacal cobaltic (II) solution, sensitivity, 0.100 μA/mm; F–J, ammoniacal cobaltic (III) solution, sensitivity, 0.200 μA/mm.

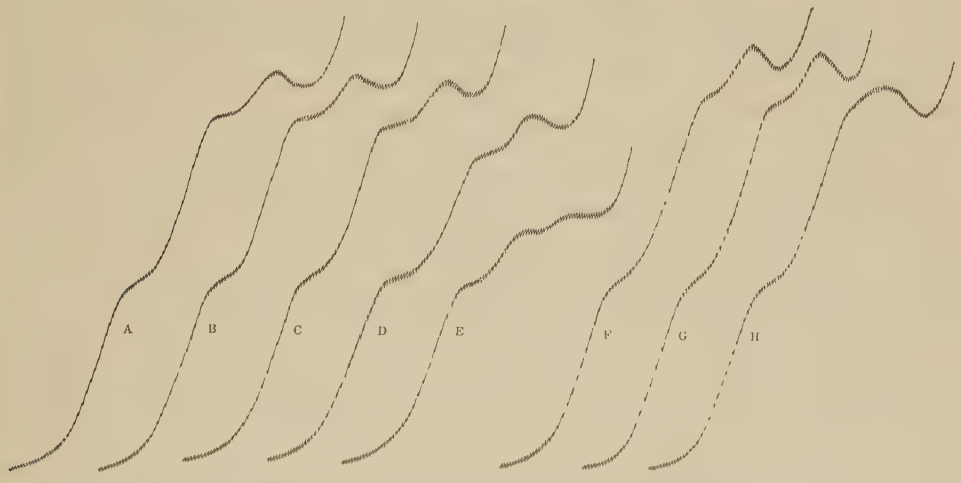


Fig. 78. Polarograms of irradiated fish meat—*Paralichthys clivaceus*.
A, F, water extractives of meat (extracted 10 times); B, irradiated A, F, 5×10^5 r; C, irradiated A, F, 1.3×10^6 r; D, irradiated A, F, 2.5×10^6 r; E, irradiated A, F, 4×10^6 r; G, water extractives of irradiated meat, 1.3×10^6 r; H, water extractives of irradiated meat, 4×10^6 r.

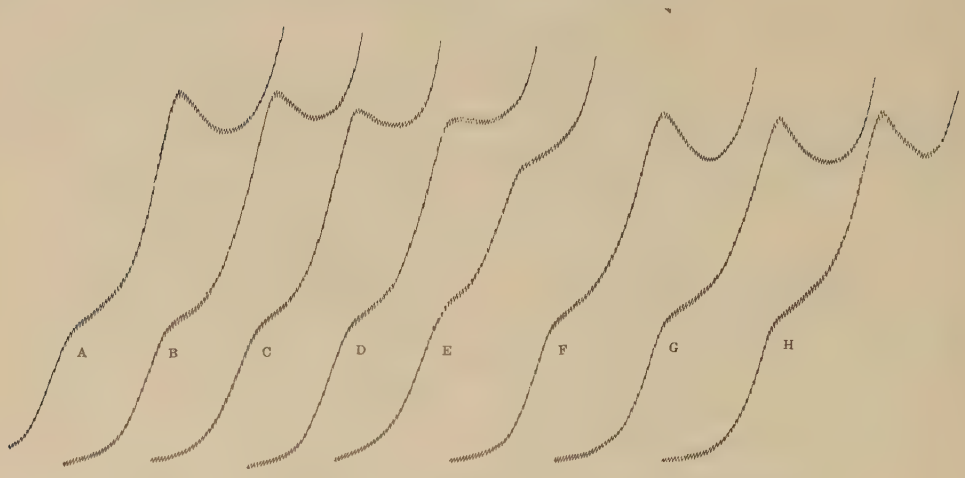


Fig. 79. Polarograms of irradiated fish meat—*Thunnus thynnus*.
F, F, water extractives of meat (extracted 10 times); B, irradiated A, F, 5×10^5 r; C, irradiated A, F, 1.3×10^6 r; D, irradiated A, F, 25×10^6 r; E, irradiated A, F, 4×10^6 r; G, water extractives of irradiated meat, 1.3×10^6 r; H, water extractives of irradiated meat, 4×10^6 r.

Table 13. Comparative observation between irradiated meat of *Thunnus thynnus* and 0.5% albumin solution.
λ max at 406 mμ; absorbancy of meat extractives ($\times$10 dil.) at 406 mμ; protein wave A, height of protein wave in ammoniacal cobaltic (II) solution; protein wave B, height of protein wave obtained by filtrate reaction technique; I, the first wave; II, the second wave of protein wave.

Sample	Meat of *Thunnus thynnus*			0.5% albumin soln.		
Irradiation dosage $\times10^4$	control	50	100	control	50	100
Color	Red	← Brownish red →		no	← slightly yellow →	
Do. water extractives	Reddish pink	← grayish brown →		—	—	—
Do. λ max at 406 mμ	1.18	1.01	0.89	—	—	—
pH	6.50	6.60	6.50	6.60	6.50	6.45
Odor	fishy odor	← irradiated odor →		no	← irradiated odor →	
Total nitrogen %	4.20	4.10	4.12	0.08	0.08	0.08
Amino nitrogen mg%	205	196	190	—	—	—
Protein wave A I	68 mm	66 mm	67 mm	62 mm	49 mm	27 mm
Protein wave A II				97	78	40
Protein wave B I	—	—	—	89	80	17
Protein wave B II	—	—	—	134	118	21

Summary

We tried the application of polarography on the studies of protein contained in muscle of aquatic animal. The following subjects were experimented by polarographic catalytic wave.

1) Specific difference of aquatic animals.
2) Comparative studies of common muscle and dark colored muscle.
3) A trial for the determination of meat freshness.
4) Denaturation of protein by γ-ray irradiation.

The main part of this paper are here summerized.

1. In the preliminary experiment the possibility of the subjects as illustrated above were clarified.

2. Detection of typical protein wave.

1) In order to obtaine a typical wave for meat extractives it is necessary to change the amount of meat extractives relative to the basic solution (ammoniacal cobaltic solution) according to the extractive ratio of meat. The extractive ratio of meat have no objection even the meat is extracted with 10, 25, 50 and 100 times of water. An adequate preparation of electrolytic solution is the following ratio of meat extractives and basic solution, 0.5 ml. of meat extractives to 20 ml. of basic solution in 10 times extractives; samely 1.0 ml. to 20 ml. in 25 times extractives and 2.0 ml. to 20 ml. in 50 and 100 times extractives.

2) For liquid material such as serum and drip of frozen whale meat, the typical protein waves are obtained in the additional rate of diluted materials (diluted with 50 times of water) and basic solution is 2.0 ml to 20 ml.

3) The pH value of basic solution is scarcely changed in the addition of meat extractives.

3. Origin of catalytic wave produced in water extractives of meat.

1) Original substance has characteristics of water soluble, and not precipitate in heating and alcohol in concentration less than 60%, and precipitate in 80% alcohol. Accordingly we conjecture the main originals due to the low-molecular protein.

2) Proteins which is precipitated by heating and treated with alcohol in concentration of 80% are the originals for the catalytic wave in a part.

3) Non-proteinous substances containing in alcoholic extractives of meat can not to be originals of protein wave though they suppress the maximum of cobaltic wave.

4. Specific difference of aquatic animals shown in protein wave.

1) Water extractives of 15 kinds of aquatic animals were compared by protein wave in ammoniacal cobaltic solution containing divalent or trivalent cobalt.

2) Reddy meat of fish such as *Thunnus thynnus* and *Katsuwonus pelamis* produces a single protein wave and white meat of fish such as *Sillago sihama*, *Limanda yokohamae* and crustacean and molluscan meat produces double protein wave.

3) All materials produce double protein wave in the basic solution containing trivalent cobalt and there is no difference in the shape of wave by the species.

4) White fish meat and crustacean and molluscan meat produce higher wave than reddy meat.

5) The different shapes of the wave have no relation to the color of muscle, because the reddy drip of frozen whale meat and serum produce double protein wave which resemble to white fish meat.

6) By the comparative observation of white meat and reddy meat, the existence of an interfering substance was ascertained in the extractives of reddy meat.

7) Polarographic characteristics of meat extractives were clarified. The relation between the height of the protein wave and the concentration of protein was same in all cases. The height of the protein wave increases first proportionally to protein concentration but tends finally to a plateau i.e. a maximal value.

8) Protein wave is observed higher in ammoniacal cobaltic solution containing trivalent cobalt than in the solution containing divalent cobalt.

5. Comparative studies of common muscle and dark colored muscle.

1) In a low concentration of protein common muscle produces a single protein wave having maximal shape and dark colored muscle produces double protein wave in the basic solution containing divalent cobalt, but in the solution containing trivalent cobalt both muscles produce double protein wave and there is no difference in the shape of protein wave between them.

2) The relation between the concentration of protein and height of protein wave in both muscles, is the same as in other aquatic animals as illustrated above. Wave hight of common muscle is higher than that of dark colored muscle.

3) Both muscles contain an interfering substance to albumin-protein wave because the double protein wave of albumin changes the shape of double protein wave to single protein wave in the addition of water extractives of meat. The contents of interfering substances are higher in common muscle than in dark colored muscle.

6. Interference of amino acids to albumin protein wave.

To clarify the origin of the different wave in the species and between common muscle and dark colored muscle, interference of amino acid to protein wave was examined.

1) Arginine, phenylalanine and tryptophane decrease the height of protein wave in the addition of their solutions, but they do not change the shape of double protein wave.

2) Histidine decreases the wave height of protein wave and also changes the double protein wave to single protein wave in addition of the solution. The change of wave is observed in a certain concentration of histidine in protein solution (ca. 2 mg%).

3) The intensity of interference of amino acids is shown as following order,

Histidine>arginine and phenyalanine>tryptophane

4) The change of the wave is observed only in ammoniacal cobaltic solution containing divalent cobalt by the addition of histidine, but it is not observed in the basic solution containing trivalent cobalt, though histidine decreases the height of protein wave.

5) The differences of protein wave showed in the species of aquatic animals and between common muscle and dark colored muscle of fish are clarified by the interference of histidine to protein wave.

7. A trial of the determination for meat freshness.

1) Increase of protein wave during the putrefaction of meat was ex-

amined compared with other test.

2) Protein wave for meat extractives in ammoniacal cobaltic solution containing divalent cobalt increases the wave during the putrefaction of meat. It does not show clearly increase in the first stage of the putrefaction but it may be possible to determine the meat freshness to present the wave height in sensitivity of μA/mm.

3) Filtrate reaction is suitable to determine the first stage of the putrefaction because the wave height cleary increases in this stage, especially in the ratio of the first and second of the double protein waves.

8. Denaturation of protein by γ-ray irradiation.

Denaturation of proteins in serum, albumin solution, globulin solution and fish meat were examined by the increase and decrease of protein wave.

1) Influence of γ-ray irradiation to serum.

i. Irradiated serum have a tinge of dark red and pigments of the serum are decomposed.

ii. The pH value does not so changed.

iii. Protein wave for serum in ammoniacal cobaltic solution containing divalent cobalt increases the wave height at the dose level of 5×10^4 r, and then decreases the height with increasing doses. Protein wave by the filtrate reaction technique increases the height with increasing doses, the change of the wave is more clearly than that of protein wave as presented above.

iv. The changes of color, odor and height of protein wave are more obviously in diluted serum than in undiluted serum.

2) Influence of γ-ray irradiation to albumin solution.

i. Change of color is not notable.

ii. The pH value tends to somewhat acidic side.

iii. Denaturation of albumin solution occurs in the irradiation dose level of ca. 8×10^5 r.

iv. Absorbancy of albumin solution in the wave length of 280 mμ increases during γ-ray irradiation.

v. Notable changes of color, odor, wave height of protein wave and absorbancy are observed in low concentration of albumin.

3) Influence of γ-ray irradiation to globulin.

i. Protein waves in the basic solution containing divalent cobalt increase with increasing dose and in higher doses more than 4×10^5 r they decrease. The change of the waves is the same as in the irradiation of ultraviolet ray.

ii. In the dose level of 1.2×10^6 r globulin is precipitated and coagulated though albumin solution is not precipitated in the same dose.

4) Influence of γ-ray irradiation to fish meat.

The denaturation of protein in fish muscle was examined in the dose of $5\times10^5\sim4\times10^6$ r by the protein wave.

i. Denaturation presented in protein wave is not so remarkable compare with the denaturation of albumin and globulin solution. We conjecture that the slightly denaturation of fish muscle may be caused by the protective substance contained in fish meat.

ii. White fish meat suffers more remarkable denaturation than reddy meat.

iii. Reddy meat have a tinge of gleyish brown because of the decomposition of red pigment.

The authors express their sincere gratitude to Prof. Drs. I. TACHI and W. SIMIZU of the Kyōto University for their invaluable advice and encourangement throughout the course of the present study. We also express their hearty thanks to Dr. I. OSAKABE and Messrs. M. SUYAMA and J. J. MATUMOTO for their helpful suggestion and criticism to the present study. We also wish to express hearty thanks to Messrs. K. FUKUSHIMA, A. ŌNUKI, K. MINOSHIMA, H. MOROOKA, S. KADOYA and K. KIKUCHI for their participation in the present study. This study was supported in part by a Grant in Aid for Departmental Scientific Research from the Ministry of Education which the offers their hearty thanks.

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Journal of the Tokyo University of Fisheries, Vol. 45, No. 2, 1959

STUDIES ON THE FAT METABOLISM OF FISH
3. RELATIONS BETWEEN FAT AND PHOSPHORUS IN RAINBOW TROUT

Toyoki Ono and Fumio Nagayama

(Received Feb. 15, 1959)

In previous paper we reported that total phosphorus, especially the soluble type phosphorus, increased rapidly in an egg of rainbow trout during the fertilization and then did gradually for 3 months until the amounts reaches to a maximum value which was represented in a month after the start of feeding, and that the fat content reached to a minimum at the hatching stage and it was constant to a certain stage called "fingerling" during 4–5 months.

From these results it was assumed that the pathway of phosphorus in the fat metabolism of rainbow trout will be closely connected with the breaking down or synthesis of fat which was not almost changed in a time of fertilization but is mainly utilized during or after the hatching.

In this paper the authors report on the pathway of each type phosphorus which fractionated in a TCA-insoluble, TCA and Ba-soluble, TCA-soluble but Ba-insoluble and lecithine type phosphorus compounds. That will be observed on the points of the relations between fat and phosphorus and the enzyme activity in concerning with the respiration, phosphorylation and other important functions in fish tissues.

Experimental

1. Material

Rainbow trout was taken as a sample on which observations were carried out at the various stages passed in fish life such as fertilization, hatching, sac-fry and fingering. Egg were picked out the ovary of adult fish which grew at Okutama Hatchery, and fertilized with the aid of sperm pressed out from a soft roe. The fertilized egg goes on the development in a pond passing such stages as above mentioned, and finally reached to a adult from larva feeling the enfluence by feeding.

These samples taken at each stage were analysed by the following methods dividing into two main components as phosphorus and fat, and were taken measure of their activity of various enzyme.

2. Method

(1) Phosphorus

The amounts of phosphate were colorimetically determined by the method of Fiske & Subbarow[1]. Before the operation of this determination, the various type phosphorus in samples were separated by the method of Le Page[2,3] to three fractions treating with TCA (trichloracetic acid) by a scheme as shown in Fig. 1.

Fraction I contains the phosphorus of acid-insoluble type, such as bone

phosphorus, the combined phosphorus with protein and lecithin type phosphorus. Fraction II, TCA- and Ba-soluble fraction, contains the available phosphorus compounds. For example, there are glucose-1-phosphate, glucose-6-phosphate, fructose-1-phosphate, phosphopyruvic acid, triose-phosphate, AMP (adenosine monophosphate), creatine-phosphate, R-5-P (ribose-5-phosphate), DPN (diphosphopyridine nucleotide=Co enzyme I), TPN (triphosphopyridine nucleotide), inosinic acid and aminoethyl phosphoric acid.

Fraction III, TCA- and Ba-insoluble fraction, contains mostly the true (actual) inorganic phosphate except a small amount of ATP (adenosine triphosphate),

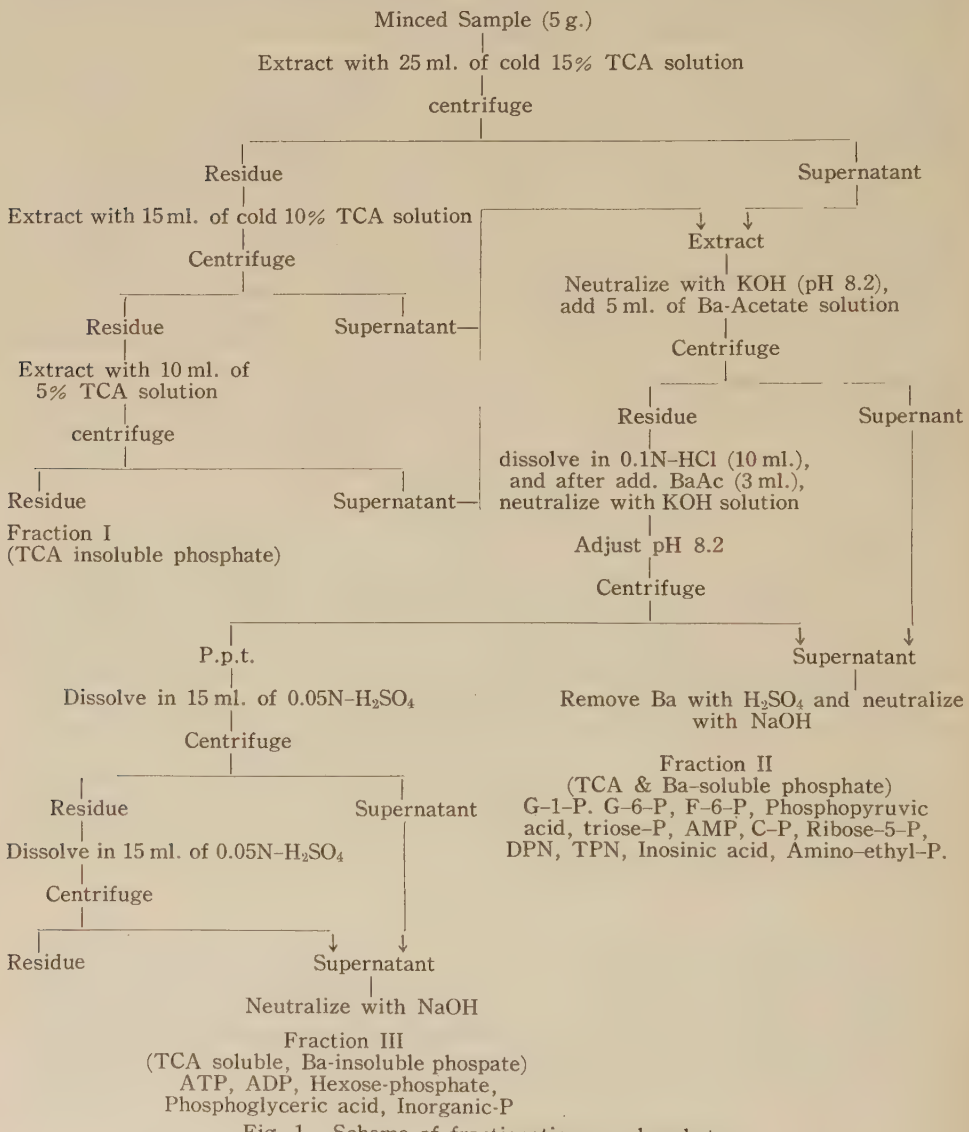

Fig. 1. Scheme of fractionation on phosphate

ADP (adenosine diphosphate), hexose-phosphate and phosphoglyceric acid.

So labile are such organic phosphates as glucose-1-phosphate and ATP, that these compounds are broken down during the treatment into inorganic phosphate and other components. Therefore, such inorganic phosphate must be also included in Fraction III as one part of total inorganic phosphorus.

The determination of phosphorus in organo-phosphorus compounds as hexose-phosphate, ATP etc., was carried on the preparations obtained from the digestion with H_2SO_4. Phospholipid phosphorus was also estimated to the digestion with H_2SO_4 by the method of Le Page.

(2) Enzyme activity

1) Succinic dehydrogenase

Enzyme preparations—Minced finely sample with ten times volume (weight) of phosphate buffer for 5 min. through a homogenizer and the supernatant was separated from mixture by centrifuging for 15 min. Though this extract contained some inactive proteins, it was used as an enzyme preparation in a result of the preliminary experiment for its enzyme activity.

The reaction mixture in estimation was made as follows.

M/15 phosphate buffer adjusted at pH 7.4.....5.0 ml.
0.01% 2-6-dichloro-indophenol aqueous solution.....2.0 ml.
0.01 M Na-succinate aqueous solution (pH 7.4).....0.2 ml.
Enzyme preparation .....1.0 ml.

After incubation of mixture for 60 min. at 20°C., the enzyme activity was estimated by the discolouration of indophenol blue using a Hilger Biochem Absorptiometer with filter 61. The enzyme activity was calculated from the equation:

$$K = -E_{61}/\text{hr.} \times 10^3$$

2) Phosphatase

Enzyme preparations were made as those of succinic dehydrogenase, and the determination of enzyme activity was carried on each type of acid and alkaline phosphatase.

(a) Acid phosphatase

The activity was estimated by the method of Azuma[4] which is a modification method of Gutman and Gutman[5]. Here substrate was sodium β-glycerophosphate which hydrolyzed more rapidly than α-compound.

The reaction mixture which possessed the following composition, was incubated at 25°C. for 30 min. and stopped its enzyme action with addition of 2 ml. of 30% trichloracetic acid solution. After filtration the solution was used to estimate the phosphate which was liberated from substrate by the enzyme action. Buffer solution was prepared from the mixture of acetic acid and Na-acetate adjusting to pH 5.7.

0.1 M β-glycerophoshate-Na salt .....1.0 ml
enzyme preparation .....1.0 ml
buffer.....1.0 ml

(b) Alkaline phosphatase

The activity of this type enzyme was determined by the method of Heppel and Morton[6] which is necessary to be presented Mg ion as an activator. The

reaction mixture was composed of 1ml. of 0.1 M β-glycerophosphate-Na salt solution (substrate), 1 ml. of 0.1 M ethanolamine-HCl buffer (pH 9.5) and 1 ml. of 0.0025 M acetate magnesium solution.

Calculation of the enzyme activity was made by the following equation from the amounts of liberated inorganic phosphates: $F=Pt/t$ mg. $\times 10^3$

(where P represents the free inorganic phosphorus, t, time for reaction in minute).

(3) Estimation of the faculty in oxygen absorption by tissue

In regard to the enzymatic oxidation of fatty acid we attempted to observe whether the egg cell of rainbow trout posses it in vitro or not. However, these experiments were all ended to a negative result with many regrets, and using a Warburg's apparatus we observed the other oxidative reactions which may be caused by the cell respiration.

To measure the oxygen consumption by tissue used the maceration juice prepared from rainbow trout egg with two times weight of M/15 phosphate buffer. Put this juice in a main compartment of Warburg's apparatus and treat at 25°C. absorbing CO_2 by KOH solution in center well. The oxygen consumption is measured by a manometer in interval of 10 min.

Results

1. Fat

In spite of the great physiological changes as fertilization, eye-opening and hatching the fat content little changed through these periods, but it diminished suddenly in a earlier stage of feeding called " fingerling ". However, in a proceeding of feeding the body fat was gradually stored in young fish (larva), and its content reached to an amount as 24% in a half year after the fertilization. (Table 1). On the other hand the weight of each sample increased accompanied with the development and growth of rainbow trout, and especially at stage No. 7, it was obvious that the protein synthesis may be much more occurred at this stage than the fat synthesis for reason of the fat decrease. Such fat consumption in this stage will be due to utilization of depot fat for the movement of larva.

2. Phosphorus

There happened in a egg of rainbow trout a peculiar physiological phenomena that the phosphorus content in fertilized egg increased for a moment after a only treating non-fertilized one with sperm. This will give a suggestion that the fertilization has a big cell division translating energy with free phosphorus which derived from fresh water.

3. Type of phosphate

As shown in Tables 1, 2 and 3 the inorganic type phosphorus diminished rapidly after the fertilization in spite of the P-increase by penetration of P-ion in egg cell. On the contrary the organic type P increased markedly at this stage compared with a non-fertilized one. Such a change on the contents of inorganic and organic type phosphorus will be provably occurred because of its translation to make the organic phosphate compounds, such as ATP, hexose-phosphate, DPN and creatine phosphate which may act on the fertilization as an active agent.

Table 1-a. Content of fat and phosphorus in rainbow trout.

(a) in individual

Stage number	1	2	3	4	5	6	7	8	9
Date of sampling	Jan. 21 Unfertilized egg	Jan. 21 Fertilized egg	Feb. 4	Feb. 20 Eyed egg	March 18 Sac fry	April 8 Fry	May 20 Fingering	June 18 Fingering	Sept. 2 Fingering
Fresh matter (g.)	0.0922	0.0989	0.113	0.105	0.103	0.156	0.340	1.38	12.7
Dry matter (g.)	0.0355	0.0343	0.0387	0.0381	0.0352	0.0314	0.105	0.319	3.88
Fat (mg.)	4.80	4.91	4.75	5.15	5.83	4.95	3.16	25.1	933
Inorganic phosphorus (μg.)									
Fraction II	2.85	2.10	5.95	1.15	1.13	3.31	7.25	65.0	812
Fraction III	40.9	33.5	9.35	8.54	52.4	98.8	117	889	6800
Total	**43.8**	**35.6**	**15.3**	**9.69**	**53.5**	**102**	**124**	**954**	**7610**
Organic phosphorus (μg.)									
Fraction II	8.65	6.06	6.25	6.95	19.0	28.4	69.3	295	4520
Fraction III	8.00	15.7	10.7	14.0	23.8	9.7	43.3	143	666
Total	**16.7**	**21.8**	**17.0**	**21.0**	**42.8**	**38.1**	**113**	**438**	**5190**
Total phosphorus (μg.)									
Fraction I	232	262	335	339	239	177	215	1190	18500
Fraction II	11.5	8.16	12.1	8.1	20.1	31.7	76.5	360	5330
Fraction III	48.9	49.2	20.0	22.5	76.2	109	160	1030	7470
Total	**292**	**319**	**367**	**370**	**335**	**318**	**452**	**2580**	**31300**
Phospholipid-P (μg.)	0.938	1.64	—	0.440	—	—	11.5	265	2260

Table 1-b. Content of fat and phosphorus in rainbow trout.

(b) in 100 parts

Stage number	1	2	3	4	5	6	7	8	9
Date of sampling	Jan. 21 Unfertilized egg	Jan. 21 Fertilized egg	Feb. 4	Feb. 20 Eyed egg	March 18 Sac fry	April 8 Fry	May 20 Fingering	June 18 Fingering	Sept. 2 Fingering
Moisture (%)	61.60	63.3	63.8	63.8	67.9	79.9	69.9	77.0	66.5
Fat (%)	13.5	14.3	12.3	13.5	16.6	15.8	3.00	78.8	24.1
Inorganic phosphorus (mg.%)									
Fraction II	8.0	6.1	15.4	3.0	3.2	10.5	6.9	20.4	21.0
Fraction III	115	97.6	24.1	22.4	149	314	110	279	175
Total	**123**	**104**	**39.5**	**25.4**	**152**	**325**	**117**	**299**	**196**
Organic phosphorus (mg.%)									
Fraction II	24.4	17.7	16.2	18.2	53.9	90.4	66.0	92.5	117
Fraction III	22.5	45.8	27.5	36.6	67.6	30.9	41.2	44.3	17.2
Total	**46.9**	**63.4**	**43.7**	**54.9**	**122**	**121**	**107**	**137**	**134**
Total phosphorus (mg.%)									
Fraction I	654	763	866	889	680	565	240	372	476
Fraction II	32.4	23.8	31.3	21.2	57.2	101	72.6	113	138
Fraction III	138	143	51.7	59.0	217	346	152	323	193
Total	**824**	**930**	**949**	**969**	**954**	**1010**	**429**	**808**	**807**
Phospholipid-P (mg.%)									
in dry matter	2.65	4.79	—	1.15	—	—	1.09	83.1	58.3
in fat	19.5	33.5	—	8.54	—	—	363	105	242

Table 2. Separation of phosphorus compounds by the LE PAGE's method.
(P mg./100 g. in dry matter)

Stage number	Total phosphorus	Soluble phosphate						Solube Total phosphorus	Total Insoluble phosphorus	Phospholipid-P
		Inorganic phosphorus			Organic phosphorus					
		Fraction II	Fraction III	Total	Fraction II	Fraction III	Total			
1	824	8	115	123	25	23	48	170	654	3
2	930	6	98	104	18	45	63	167	763	5
3	949	16	24	40	16	28	44	84	866	—
4	969	3	22	25	18	37	55	80	889	1
5	954	3	149	152	54	68	122	274	680	—
6	1010	11	314	325	91	32	123	448	565	—
7	429	7	110	117	66	42	108	225	204	1
8	808	20	279	299	93	44	137	436	372	83
9	807	21	175	196	117	172	135	331	476	58

Table 3. Distribution of phosphate in various types. (Ratio)

		Stage number								
		1	2	3	4	5	6	7	8	9
	TCA insol. phosphorus / Total phosphorus (%)	79	82	91	92	71	56	48	46	60
	TCA soluble-P / Total-P (%)	21	18	9	8	29	44	52	54	40
Ratio	Total-P in Frac. II	19	14	38	27	21	23	32	26	42
Ratio	Total-P in Frac. III	81	86	62	73	79	77	68	74	58
	TCA soluble inorg-P / Total-P (%)	15	11	4	2	16	32	27	37	24
	Inorg.-P in Frac. II	7	6	39	11	2	3	6	7	11
	Inorg.-P in Frac. III	93	94	61	88	98	97	94	93	89
	TCA soluble org.-P / Total-P (%)	6	7	5	6	13	12	25	17	16
	Organic-P in Frac. II	52	28	37	33	44	74	61	68	86
	Organic-P in Frac. III	48	72	63	67	56	26	39	32	14

A little attention, however, must be paid for the amounts of total phosphates, because the inorganic phosphates involve a little amount of inorganic ones which are made in a result of hydrolysis by treatment of the labile organic compounds especially glucose-1-phosphate.

4. Succinic dehydrogenase

In this experiment the enzyme activity showed less highly at a earlier stage of development, but it indicated a considerable higher value at a stage of hatching as shown in Table 4. It was obvious that such enzymes concerning with oxidation or reduction in living cell may be much more secreted at larva stage than egg one.

Table 4. Succinic dehydrogenase activity of rainbow trout.
$K = -\Delta E_{61}/hr. \times 10^3$

M/15 Phosphate buffer at pH 7.4 5 ml., 0.01% 2.6-dichloroindophenol 2 ml., 0.01M Na-succinate 0.2 ml, enzyme preparation 1.0 ml.

Stage number	*K*/ml.	*K*/mg. in dry matter	*K*/mg. in tissue	*K*/Individual
1	0	0	0	0
2	19.3	0.60	0.21	19.3
3	5.8	0.13	0.33	32.2
4	30	0.83	1.47	166
5	—	—	—	—
6	200	7.7	2.28	357
7	58	6.9	0.85	290
8	136	9.7	1.69	234
9-muscle	9.0	1.0	0.42	
9-viscera	24.0	2.5	0.91	

5. Oxygen adsorption by living cell

As shown in Table 5 and Fig. 2, the oxygen absorption by living cell rose with a proceeding of development. This shows distinctly that a strong physiological function appeared remarkably for the activation of respiration, oxidation of fatty substances and movement of larva, and the oxygen will be used to produce energy for its necessity.

Table 5. Oxygen absorption by tissue of rainbow trout.

Stage	O_2-uptake μl./hr.		Ratio
	In 2 ml. of tissue solution	In individual	
1. Unfertilized egg	57.1	8.57	100
2. Fertilized egg	101	15.1	176
3. 〃	114	17.1	200
5. Hatching	153	23.0	268

6. Phosphatase

The activity of enzymes in this group was not noticiably high value in this experiment as well as the other enzymes. Through all times, the high activity of enzyme was made by the alkaline type enzyme only at earlier stage of development, in a unfertilized egg, and the lower activity was represented in a larva, a latter stage of development (Table 6).

Discussion

The role of phosphorous in fat metabolism have been not resolved in this experiment. However, it was very interested in the observations that the content of phosphorus especially organic type phosphous increased rapidly in a fertilized egg but decreased in inorganic type one in opposition to less changes in fat

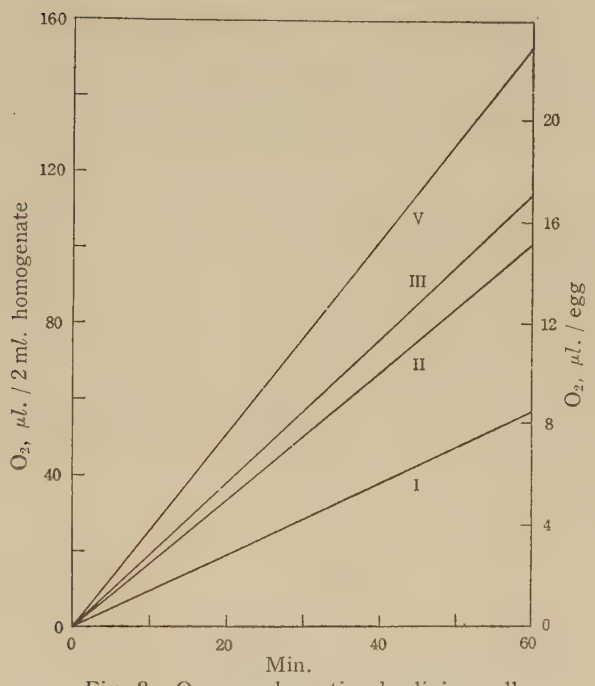

Fig. 2. Oxygen absorption by living cell.
Roman numerals represent stage numbers.

Table 6. Phosphatase activity of rainbow trout.
$F = \Delta P_t$ (ug)/t (min.) / mg. of dry matter
(where P represent the inorganic phosphorus liberated by enzyme; t, time on reation in hour).

Stage number	Enzyme preparation mg./ml.	Acid phosphatase		Alkaline phosphatase	
		ΔP_{30} μg./ml.	$F \times 10^3$	ΔP_{30} μg./ml.	$F \times 10^3$
1	6.3	12.0	63	52.4	279
2	5.8	24.2	139	29.4	169
3	5.9	23.2	131	—	—
4	—	—	—	—	—
5	—	—	—	—	—
6	12.8	63.2	165	8.9	23
7	14.7	18.8	42	5.9	13
8	24.0	54.1	62	20.5	24
9-Muscle	12.0	26.6	74	20.5	57
9-Viscera	25.0	27.6	37	17.8	17
Buffer		Acetic acid+Na-Acetate (pH. 5.7)		Ethanolamine-HCl (pH 9.5)	
Substrate		β-Glycero phosphate-Na-salt		β-Glycerophosphate-Na-salt	
Temp.		25°C.		25°C.	
Activator		—		Mg-acetate	

content. These facts gave us an explanation that the phosphate makes its content to rise during the fertilization must be introduced into egg cell by osmotic pressure from the outside of egg, fresh water, and that phosphate may be utilized for the synthesis of organic phosphorus compounds, such as ATP, adenylic acid and glucose-1-phosphate which are available to take place perfectly the physiological functions.

On other hand the lipid had little change on its content as earlier stage during development, therefore it will be less utilized to such functions as above mentioned. However, while the lipid content was soon diminished at a larva stage, there will be a difference on physiology between both stage.

We can explain for this expending question that the lipid is a necessary material to perform the growth in such a period as "fingering", while the organic-phosphorus compounds are much more needed than lipid at a earlier stage of development. Passing this period a adult fish must be finished when a normal growth will be carried out by the synthesis of fat, protein and other available matters in body.

Summary

1. Studies on the relations between fat and phosphorus in rainbow trout were carried on taking the sample at 9 stages during 8 months.

2. Lipid did not change on its content a earlier stage but it diminished rapidly after the hatching. However, the storage of lipid was recognized in all tissue of body at a latter feeding stage. On the contrary the phosphate increased in egg cell immediately after the fertilization, and the type of increased phosphate was not inorganic compound but organic one.

3. Succinic dehydrogenase showed generally less high activity in this experiment except that of hatching stage at which was observed a considerable high activity.

4. Oxygen absorption by living cell in egg of various stages rose with proceeding of the development of fish. Both acid and alkaline phosphatase did not give a noticiable activity.

The authors gratefully acknowledge the kind supplement of sample given by Mr. Tarō Matsumoto, Director, and Mr. Tsumoru Masuda, chief of the Okutama Hatchery, Tokyo Metropolitan Fisheries Experimental Station. We also wish to thank Mr. Shinichi Tsukada and Mr. Shigeru Tomomatsu for their assistance in performing the phosphorus analysis reported in this paper.

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Journal of the Tokyo University of Fisheries, Vol. 45, No. 2, 1959

ENZYMATIC STUDIES ON THE GLYCOLYSIS OF FISH MUSCLE II. COLORIMETRIC METHOD FOR DETERMINATION OF SUGARS IN MUSCLE

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(Received Jan. 16, 1959)

Various colorimetric methods have been developed and widely used for the determination of sugars. The reagents, such as resorcinol[1] cysteine-carbazole[2], diphenylamine[3] and indole[1] for ketohexoses; carbazole[5], cysteine-sulfuric acid[6], anthrone[7], arsenomolybdate[8] and ferricyanide[9] for total hexoses; orcinol[10] and cysteine-sulfuric acid[11] for pentoses, are well-known examples for colorimetric analysis of sugars. The colorimetry is more predominant in its sensitivity than other methods such as titrimetry. There is no complication in the determination of a single sugar system or separated sugar, but with some colorimetic method it is not able to avoid a considerable error with the complex system as the extract of bio-materials.

Arsenomolybdate reagent is most commonly applied in biochemical analysis, as it is inexpensive and very sensitive. However, arsenomolybdate reaction is found to be not rigidly specific to hexoses. This nonspecificity is a defect when the sample solution for analysis contains two or more sugars, but it may be possible to make it a merit that several sugars are simultaneously determined with one reagent if each sugar are separated by suitable method.

There are found two types of free sugars in muscle extract; the one is glucose occurred from glycolysis, and the other ribose from decomposition of nucleic substances. Moreover, the extract contains a considerable amounts of free amino acids. There are often obscure results in sugar analysis on muscle extract using arsenomolybdate method.

Present paper will report on the examination of arsenomolybdate reaction with various sugars and other compounds to establish the adequate procedure of sugar analysis, and will also refer to the method for quantitative analysis of glucose and ribose in muscle extract.

Experiments

I. Reagents and methods

Reagents and methods used here followed to Hestrin *et al.*[12]

Somogy's copper regent[13]: (a) 10% copper sulphate ($5H_2O$); (b) in 700 ml. of water 28g. of anhydrous dibasic sodium phosphate is dissolved, and 40g. of sodium potassium tartarate ($4H_2O$) is added. After solution is complete, 100 ml.

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of 1N sodium hydroxide and 120g. of anhydrous sodium sulfate are added and volume is made up to 900 ml. with water. After 2 days sediment is filtered off. At the use, 1 volume of (a) is mixed with 9 volumes of (b).

Nelson's arsenomolybdate reagent[14]: in 450 ml. of water 25 g. of ammonium molybdate ($4H_2O$) is dissolved and 21 ml. of concentrated sulfuric acid is added. The solution is mixed with the solution of disodium arsenate (3 g. in 25 ml. of water), and incubated for 24 hours at 37°.

Precipitating reagent: (a) 45 g. of barium hydroxide is dissolved in 600 ml. of water; (b) 5% solution of zinc sulfate ($7H_2O$).

Method: to 5 ml. of sample solution containing sugars and protein, 1 ml. each of Ba- and Zn-solution. Precipitate is centrifuged or filtered off, and washed with minimum water. Suitable aliquot of solution is added in a final volume of 7 ml. to a test tube containing 2 ml. of copper reagent and heated in boiling water bath for 20 minutes, and cooled to room temperature in tap water. To the tube, 2 ml. of arsenomolybdate reagent is added and volume is made up to 15 ml. with water. Intensity of blue colour developed is measured by Hilger Biochem Absorptiometer using filter 52 (520 mμ) and cuvette of 1 cm. depth.

Orcinol method for ribose and chromatographic technique for sugar separation will be noted in the section of results.

II. Results

A. Examination on the arsenomolybdate method.

Volume of reaction mixture. Colour intensity varies considerably with the constitution of the reaction mixture or the concentration of reagents. When sample solution and arsenomolybdate reagents are constant in their volumes, the colour intensity is proportional to the volume of copper reagent; while that is proportional to the volume of arsenomolybdate reagent when sample and copper reagent are constant. On the contrary the intensity decreases with increase of sample solution when volumes of arsenomolybdate and copper reagents are constant (Table 1).

Table 1. Effect of constitution of reaction mixture to colour intensity.

Sample	Glucose	Copper reagent	Arseno-molybdate reagent	E_{520}		
				Test	Blank	T–B
ml.	μg.	ml.	ml.			
5	90	1	2	.295	.035	.265
5		2	2	.451	.170	.282
5		4	2	.490	.208	.282
5	103	2	1	.393	.090	.303
5		2	2	.413	.090	.323
5		2	4	.403	.088	.315
1	90	2	2	.428	.090	.338
2		2	2	.430	.111	.319
5		2	2	.381	.099	.282
7		2	2	.338	.069	.269

Hestin *et al.* established volumes of sample, copper and arsenomolybdate reagents as 2 ml. each, but it may be permitted to alter the volume of sample solution as 5 ml.

Reaction time. The examination on the reaction time of copper reagent with glucose in boilling bath shows that the heating process for 20 minutes is necessary and enough to colour development (Table 2).

Stability of colour. At 2, 5, 10, 15, 30 and 60 minutes after the time of addition of arsenomolybdate reagent followed by making up volume to 15 ml., the colour intensity is measured (Table 3). It is found that the colour is stable for long period after the time of development, even for 24 hours or more. This excellent stability is convenient in the simultaneous analysis of many samples.

Table 2. Effect of reaction time of copper reagent with glucose. (100 μg.)

Time	E_{520}		
	Test	Blank	$T-B$
min.			
5	.359	.073	.286
10	.398	.086	.312
15	.408	.090	.318
20	.416	.096	.320
30	.416	.096	.320

Table 3. Stability of colour. (100 μg. of glucose)

Time	E_{520}		
	Test	Blank	$T-B$
min.			
2	.402	.070	.337
5	.399	.087	.312
10	.399	.088	.311
15	.400	.092	.311
30	.405	.093	.312
60	.411	.100	.311

Light absorption at different wave length. Light absorption at different wave length shows the minimum at 520 mμ (filter 52) with Hilger Absorptiometer. This wave length is advantageous to measure the colour because the effect of blank test is also minimum as Ashwell[16] postulated previously (Table 4 and Fig. 1).

Table 4. Light absorption at different wave length. (105 μg. of glucose)

Filter No.	Wave length	E_{520}		
		Test	Blank	$T-B$
	mμ			
43	430	.680	.238	.442
47	465	.640	.174	.466
49	490	.452	.120	.332
52	520	.404	.078	.326
55	545	.467	.078	.389
58	580	.707	.118	.589
61	610	.810	.124	.686
70	680	1.10	.178	.922

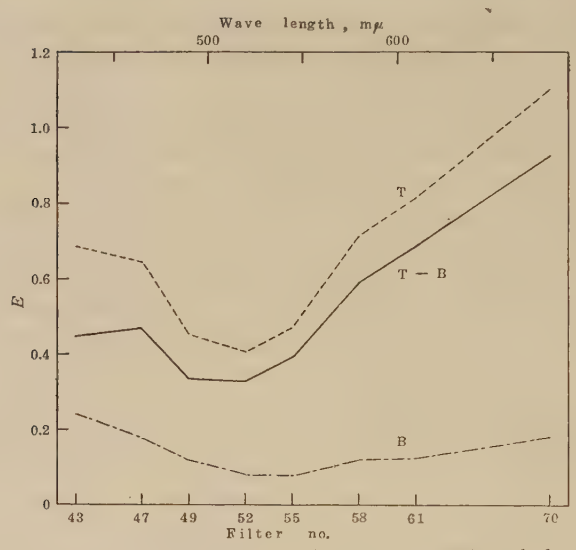

Fig. 1. Light absorption at different wave length by the reacted solution of glucose with arsenomolybdate reagent. (105 μg. of glucose).

Reaction with sugars and other compounds. It was often observed that the analytical value of sugar by arsenomolybdate method was lesser with muscle extract which is pretreated with ion exchange resins or chromatography than the nontreated one. To know the cause of discrepancy in those analytical results, colour reactions of arsenomolybdate reagent with various sugars and other compounds were observed (Table 5). Among hexoses, fructose is most positive to

Table 5. Colour yields with sugars, amino acids and other compounds. (Ratio of E_{520} to glucose in %)

Compound	Mol. eq.	Wt. eq.	Compound	Mol. eq.	Wt. eq.
Arabinose	65	78	Arginine	−1	−1
Ribose	60	69	Cysteine	27	24
Xylose	86	103	Glutamate	−2	−2
Glucose	100	100	Glycine	0	0
Mannose	90	90	Histidine	2	3
Galactose	81	81	Leucine	−0	−0
Fructose	110	110	Lysine	0	0
Sucrose	0	0	Phenylalanine	3	3
Maltose	57	29	Proline	3	5
Lactose	108	54	Tryptophane	14	13
Glycogen	0	0	Tyrosine	16	16
Acetaldehyde	0	0	Valine	−0	−1
Formaldehyde	0	0	Asparagine	−2	−2
Glucose-1-P	12	5	Glutathione	30	16
Alanine	3	7	Ascorbic acid	73	75

the reaction. Pentoses and disaccharides except sucrose are also found to be positive, while glycogen, acetaldehyde and formaldehyde are negative. Among amino acids, cysteine, tryptophane and tyrosine react with arsenomolybdate reagent positively to form blue colour as well as sugars. Effects of alanine, histidine, phenylalanine, lysine and proline are considered as negligible. Arginine, glutamate, leucine and valine are negative or slightly inhibitory. Glutathione is also positive, and ascorbic acid is very effective. Deproteined muscle extract may contains not only free glucose and ribose, but also various free amino acids. Therefore, developed colour with arsenomolybdate method does not give the amounts of sugars exactly in the analysis of muscle extract.

Techniques for removal of interfering substances and for separation of individual sugars will be noted in following sections.

Effect of precipitating reagents. Tricloracetic acid inhibits completely colour development even after the treatment with ion exchange resins. Lead subacetate is very effective precipitating reagent, but at the use of lead subacetate numerous adsorption of sugars on the precipitate occurs, and colour development is inhibited. Thus, these two acetate reagents can not be used. Ba-Zn reagent also brings about the adsorption of sugars on precipitate, but sugars are recovered completely by washing of precipitate with minimum water. However, excess of this reagent in solution inhibits colour development (Table 6).

Table 6. Effect of Ba- and Zn-solution to colour development.

	E_{520}
Glucose (105 μg.)	.412
Glucose+Ba-soln.	.200
Glucose+Zn-soln.	turbid*
Glucose+Ba-Zn-soln.	.077
Blank	.087

* Colour intensity unchanged

Calibration with the standards of glucose and ribose. Under the conditions postulated above, the calibrations with standards of glucose and ribose were

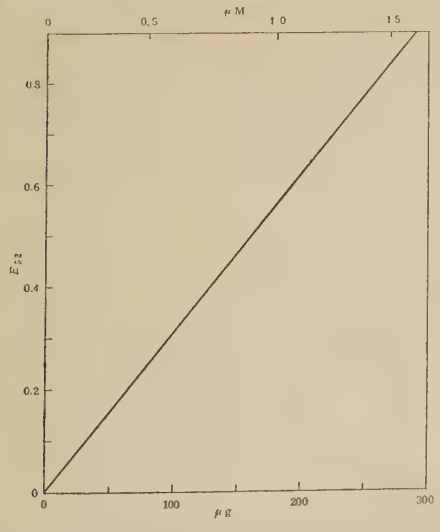

Fig. 2. Relation between the concentration of glucose and color intensity in arsenomolybdate method.

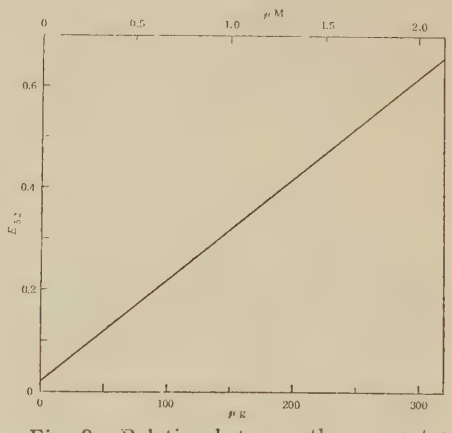

Fig. 3. Relation between the concentration of ribose and colour intensity in arsenomolybdate method.

obtained as shown in Figs. 2 and 3. Equations for calculations of sugar concentrations are as follow:

$$X_g = 323\, D_{52} - 1.29\,, \qquad X_g' = 1.80\, D_{52} - 0.007$$
$$X_r = 507\, D_{52} - 12.1\,, \qquad X_r' = 3.41\, D_{52} - 0.081$$

where X_g represents μg.; X_g' μM of glucose; X_r, μg.; X_r', μM of ribose; D_{52}, optical density at 520 mμ. Most desirable ranges of sugar concentrations are as follow: 50—150 μg. (0.28~0.84 μM) for glucose; 70—220 μg. (0.78~2.44 μM) for ribose, respectively.

Although the calibrations are shown in this paper with only glucose and ribose, other sugars such as fructose may be determined by individual calibration.

B. Determination of free glucose and ribose in fish muscle.

Extraction of free sugars from muscle. There have been reported that 95% alcohol is suitable solvent for extraction of free sugars from fish muscle[16]. However, there was found no difference among 95, 50% alcohol and water in the recovery test for sugars from muscle. Alcohol does not extract polysaccharides such as glycogen, but extracts some fatty substances which often inhibit following analysis. Thus, water may be used for extraction of free sugars, although it may extracts water soluble compounds other than free sugars. Volume of water was decided as 10 volumes of sample from the experimental result shown in Table 7.

Removal of amino acids and other interfering substances. To avoid the influences of amino acids and other interfering substances to the colour reaction,

Table 7. Effect of volume on sugar extraction from muscle with water. Extract from 2 g. of muscle is made up volume to 50 ml., and measured.

Volume of water for extraction	E_{520}
ml.	
30(10+10+10)	.251
20(10+5+5)	.254
20(10+10)	.250
15(10+5)	,216

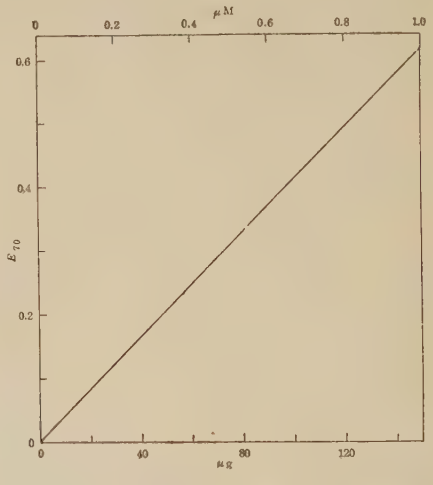

Fig. 4. Relation between the concentration of ribose and colour intensity in orcinol method.

it is desirable to remove previously these substances which occur in solution to be analysed. For that purpose, Dowex 50×2 (mesh 50–100) and Duolite A 4 may be used. There two resins do not adsorb any free sugars. A simple column technique seems to be more effective than batch technique. About 70 ml. of the extract which is obtained from 5 g. of muscle and is deproteined by Ba-Zn solution is passed through the column (ca. 1 cm. dia.) contains 12 ml. of Dowex

50 by the speed of 1/4 bed volume per minute. Column is washed with 4 bed volumes of water. Combined solution is then passed through the column of Duolite which is prepared similarly. Final volume of solution passed through the columns of resins is made up to 200 ml. with water. The solutions contains no interfering substances and is ready to be analysed for total free sugars, in general, glucose and ribose.

Resins must be regenerated by washings after the use: column of Dowex 50 is washed with 2 bed volumes of 5% hydrochloric acid and 20 bed volumes of water by the speed of 1/4 bed volume per minute; Duolite A4 with 2 bed volumes of 5% sodium hydroxide and 20 bed volumes of water.

Correction by orcinol reaction.[10]. As orcinol reaction is found to be comparatively specific to pentose (Table 8), the analytical result on the mixture of glucose and ribose with arsenomolybdate method will be corrected briefly for glucose concentration by the determination of ribose by orcinol method[10].

Table 8. Orcinol reaction with sugars.
(Ratio of E_{680} to ribose in %)

Sugar	E_{680}		Colour
	Mol. eq.	Wt. eq.	
Arabinose	76	76	Green
Ribose	100	100	Green
Xylose	90	90	Green
Glucose	4	3	Brownish green
Mannose	8	6	Brown
Galactose	7	5	Brown
Fructose	8	7	Reddish brown

To 3 ml. of sample solution, 3 ml. of 0.1 % $FeCl_3$ (in HCl) and 0.3 ml. of 10% alcoholic orcinol solution are added, and heated in boilling bath for 30 minutes. After cooling, volume is made up to 10 ml. with water, and green colour developed is measured at 680 mμ (filter 70). Calibration was obtained as shown in Fig. 5, and equation is as follows:

$$X_r = 244\, D_{70}\,, \qquad X_r' = 1.63\, D_{70}$$

where X_r represents μg.; X_r', μM of ribose.

However, hexoses including glucose are not completely negative to this reaction as shown in Table 8. Therefore, correction by orcinol method is not perfect.

Separation of sugars by paper chromatograpy. To separate sugars, paper chromatography is supposed to be most reliable technique, although it requires some skill and is time-consuming. A method of Shallenberger *et al.*[17] was applied in this experiment.

Deionized sample solution is concentrated under reduced pressure to contain above 30 mg. of sugars per ml. Deionization process is necessary because amino acids often causes a considerable loss of free reducing sugars by condensation during concentration. Toyo filter paper No. 50 washed with diluted acid is cut into strips of 3 cm. width. Ten μl. of concentrated sample solution containing

about 100 μg. of each sugar is pipetted to the starting line as narrow zone, and developed for 18–24 hours with *n*-butanol-acetic acid-water (4 : 1 : 5 or 4 : 1 : 2). Tests for standards of sugars should be paralelled. After air-drying, the strips of standards and one of unknown sample are sprayed with aniline-oxalate reagent (1 ml. of 50% alcoholic aniline solution is mixed with 35 ml. of 0.2 M oxalic acid), and heated for 7 minutes at 105°. Glucose appears in brownish zone, and ribose in pink. Strips of sample which are not treated with spraying reagent are cut in 3 cm. width at the part corresponding to coloured zone. Pieces of paper (3× 3 cm.) are extracted with 10 ml. of water being warmed to 80° for 10 minutes to elute sugars[18]. Eluate is determined its sugar content with arsenomolybdate method.

Rf values for glucose and ribose are shown in Table 9. As there are found slight differences in Rf values between standards and sample, it is always neces-

Table 9. Rf of glucose and ribose.

n-Butanol-acetic acid-water	Sample	Rf	
		Glucose	Ribose
4 : 1 : 2	Standard	0.32	0.52
	Extract	0.37	0.59
4 : 1 : 5	Standard	0.14	0.22
	Extract	0.17	0.25

sary to carry out the colour detection with standards and sample developed on paper strips. Recovery was found to be about 100%. Fructose was not detected on chromatogram with muscle extract, and is supposed to be not present as free sugar in muscle.

Summary

1. For the determination of glucose in muscle extract, arsenomolybdate method, orcinol method and paper chromatographic technique were examined.

2. Arsenomolybdate reaction is not rigidly specific to hexoses, devoloping blue colour not only with hexoses but with pentoses and amino acids, etc.

3. Interfering substances, such as amino acids, metal ions, could be removed with ion exchange resins, Dowex 50 and Duolite A 4.

4. Arsenomolybdate analysis could be corrected briefly for glucose with the determination of pentoses by orcinol method, but this correction was not perfect in the analysis of muscle extract.

5. Glucose and ribose were only sugars in muscle extract which were found in free type, and free fructose was not detected.

6. Glucose and ribose may be determined quantitatively with arsenomolybdate method after paper chromatographic separation.

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Journal of the Tokyo University of Fisheries, Vol. 45, No. 2, 1959

STUDY ON THE FISH-GATHERING EFFECTS OF AIR CURTAIN

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(Received Feb. 16, 1959)

The author has reported about the *Trachurous japonicus* to air bubble on another occasion. Essentials of this report show that *Trachurous japonicus* are usually watchful and afraid of air bubble. However, this disposition of the fish not only retires but become even reversed as they stay long in the neighborhood of air bubble. Report of Busse-Bay Fisheries Laboratory U. S. A. 1955 reported that the *Clupea pallasis* were effectively gathered by means of air curtain in field experiments. The authors experimented on the effects of air curtain in gathering *Trachurous japonicus*.

Arrangement of experimental set and method

A concrete tank of the following size was used; 4.5 m. in length, 1.5 m. in breadth and 0.8 m. in depth. The amount of water was adjusted to be 60 cm. in depth. Experimental room was made dark. Three white lamps (100 *volts* 200 *watts*) as light source. Under water illumination was measured by an under-water lux meter of Murayama's type which was kept horizontal on the tank bottom.

The measured strength of illumination are shown in Fig. 3. The air pipe used was made of brass; diameter 5.5 cm., 150 cm. in length and provided with 27 holes evenly distributed intervals of 5 cm. The bubbles were supplied from a propane gas generator. A vinyl tube fitted to the cock of the propane gas-vessel was connected at the other end to the above brass pipe, was set as shown in Figs. 1 and 2. Air were discharged in bubble continuously through the 27 air holes of the brass pipe. The arrangement to tow the brass pipe is shown Fig. 1.

As test fish, 50 individuals of *Trachurous japonicus* were provided in total.

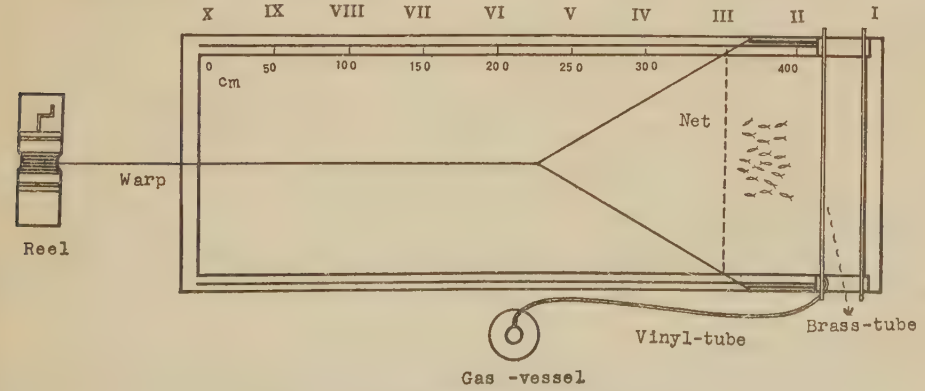

Fig. 1. Arrangement of the experiment.

Every 20 of them was used in every run. Their average body length was 14 cm. Water temperature was kept at 13°～13.8°C. Test fish were first enclosed in section

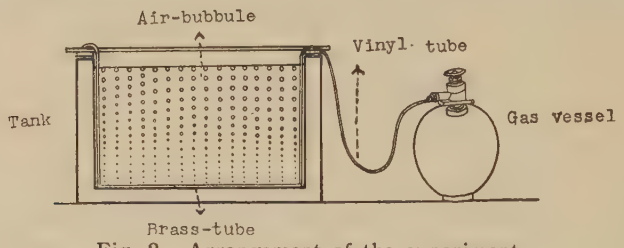

Fig. 2. Arrangement of the experiment.

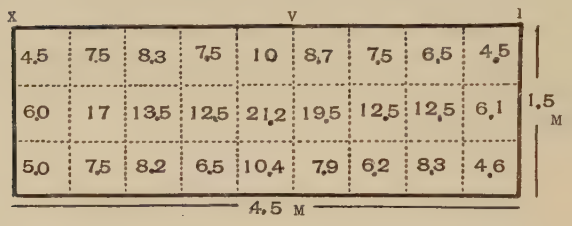

Fig. 3. Illumination (lux). Into the tank.

I with a net allowed to swirl for five minutes. The net was then removed out of the tank with simultaneously stirting a continuous discharge of air bubble from every holes and setting the fish free. The brass pipe was drawn at a speed of 6.7 cm./sec.

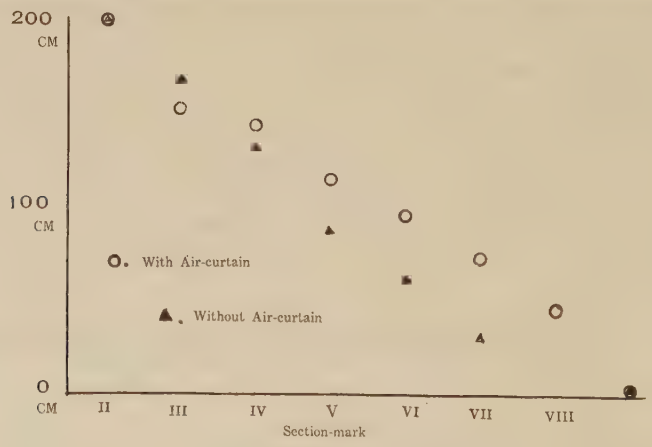

Fig. 4. Distance, from section-mark to fish.

Preliminary experiment

In this case, there were carried out 20 runs, with the pipe only; (with no gas bubble.)

Experiment proper

In this case, the gas was discharged in bubbles evenly through 27 air holes of the pipe so that an air curtain was bult up there, there were carried out 20 runs.

Result of experiment

Behavior of fish

In the preliminary experiment, the fish rushed at once for the open side on removal of the enclosing net, some of them returning soon to the original area. (The brass pipe was made a start from section I when the fish passed the section IV. Relation between the fish-position and the pipe's position was shown Tables 1 and 2. Tables 1 and 2 were shown the distance from the center of fish-group to every section marks when the brass pipe or air curtain was reached to every marks.) This tendency of comming back was observed especially in the VII and VIII sections. The fish attempting to return were a little confused when faced with the brass pipe in motion but become

Table 1. Preliminary experiment (without air curtain).

Exp. times \ Section mark	II	III	IV	V	VI	VII	VIII	IX
	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
1	200	150	95	70	50	5	0	0
2	200	140	70	50	30	0	0	0
3	200	115	110	65	60	0	0	0
4	200	150	100	75	75	45	0	0
5	200	195	140	80	55	45	30	0
6	200	190	165	90	50	60	0	0
7	200	222	180	135	50	50	0	0
8	200	195	165	100	55	20	0	0
9	200	175	120	85	60	5	0	0
10	200	170	100	75	60	20	0	0
11	200	110	90	90	65	32	0	0
12	200	200	140	110	87	60	0	0
13	200	150	150	90	55	25	0	0
14	200	150	115	70	50	100	0	0
15	200	110	70	40	50	20	0	0
16	200	200	150	105	50	20	0	0
17	200	185	170	50	50	5	0	0
18	200	175	160	130	85	50	0	0
19	200	180	150	140	85	20	0	0
20	200	220	180	140	115	60	15	0
Σ	4.000	3.382	2.620	1.790	1.237	642	45	0
M	200	169.1	131	89.5	61.4	32.1	2.3	0

* This table was shown that the distance from every section-mark to the group of the testing fish, when the brass tube was reached to every section-mark.
* The brass tube was made a start from the section-mark I when the group of the testing fish passed to the section-mark IV.

gradually unmindful of it so that they readily swam over this pipe especially in the cases of section III~VII, as shown Table 1.

In the experimental proper, behavior of the fish was similar to that observed in the preliminary experiment. But the fish become crowded entirely in sections VIII and IX when they in the early time of this experiment were faced with the air curtain. All the fish were found crowded in the corner of the thank wall in the later stage of this experiment. The fish gathered around a point in section IX when the air curtain reached section VII or VIII, as shown in the Table 2.

Table 2. Experiment (with air-curtain).

Exp. times \ Section mark	II	III	IV	V	VI	VII	VIII	IX
	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
1	200	200	185	150	120	80	40	0
2	200	150	170	140	120	75	47	0
3	200	180	150	140	120	75	45	0
4	200	150	140	100	70	60	30	0
5	200	120	110	80	80	60	32	0
6	200	110	110	100	70	65	45	0
7	200	165	135	100	70	60	38	0
8	200	150	120	80	75	60	40	0
9	200	150	120	90	70	60	38	0
10	200	180	170	135	120	90	50	0
11	200	150	150	150	120	85	60	0
12	200	120	145	100	100	70	60	0
13	200	180	120	115	90	80	50	0
14	200	120	130	120	100	70	50	0
15	200	130	135	105	95	70	40	0
16	200	150	145	135	120	90	45	0
17	200	195	170	135	110	90	50	0
18	200	160	170	150	130	90	50	0
19	200	120	120	110	90	70	50	0
20	200	150	125	90	50	50	43	0
Σ	4.000	3.030	2.820	2.325	1.920	1.450	893	0
M	200	151.5	141	116.3	96	72.5	44.2	0

* This table was shown that the distance from every section-mark to the group of the testing fish, when the air-curtain was reached to every section-mark.

* The air-curtain (brass tube) was made a start the section-mark I when the group of the testing fish passed to the section-mark IV.

The fish thus first compelled to crowd in the corner of the tank by the air curtain swam over this air curtain in the long run. The positions of the fish, when the fish swam over the air curtain, was shown in Table 3.

Table 3. Positions of the fish when were overed and run to the brass-tube or air-curtain

Exp. times	Kinds of Exp.		Exp. times	Kinds of Exp.	
	without air curtain	with air curtain		without air curtain	with air curtain
	cm.	cm.		cm.	cm.
1	145	40	11	100	40
2	150	40	12	110	40
3	150	47	13	130	43
4	100	40	14	154	40
5	70	45	15	102	47
6	100	44	16	130	40
7	110	40	17	140	40
8	140	47	18	125	45
9	145	43	19	148	40
10	120	40	20	90	40
			Σ	2.459	841
			M	123	42

Conclusion

In preliminary experiment, position of the pipe at the time when the fish dared to swim over it is read as 123 cm. (the corresponding mark standing in section VII) in average. In experiment proper, position of the air curtain at the time when the fish crossed over it was find to be 42 cm. (in section IX) in average. The difference between the position of the dormont air bubbler and that of the air curtain was looked on as the true efficiency of air curtain in driving the fish, corresponding to about 81 cm. in the present case.

The difference between tables 1 and 2 was shown the degrees of watchfulness by fish. These data clearly indicated that the air curtain makes an effectual tool for driving the *Trachurous japonicus*. However, the air curtain is not possible to keep the fish for a long time in a desired place.

Acknowledgement

The authors thanks the students for their kindness shown in making this experiment.

Journal of the Tokyo University of Fisheries, Vol. 45, No. 2, 1959

STUDY ON THE DISPOSITION OF FISH TOWARDS LIGHT NO. 3 THE STRENGTH OF ILLUMINATION COMFORTABLE TO *COLOLABIS SAIRA*

Yutaka Imamura and Atsushi Koike

(Received Feb. 16, 1959)

Cololabis saira are caught with stick-held dip net when the fish are tempted by light togather in crowd. Fishermen have neither studied the behavior of *Cololabis saira* towards light nor learned what strength of illumination pleases *Cololabis saira*. At the present time, this manner of fishing is practised on a keen cometitive basis in the direction of using the light ever increasing in power.

The authors experimented on the behavior of *Cololabis saira* towards light and the strength of illumination which *Cololabis saira* is fond of, the results being reported in the descriptions below.

Outline of the fish-gathering light for fishing

The authors examined fishing-light of the fishing boats which entered the fishing port of Kisenuma in Miyagi prefecture in the year 1957. These boats were classified for convenience sake into 7 groups according to the tonage. Results of examination are shown in Table 1. The authors calculated the illumination lux for boats of different group each.

Table 1. Arrangements of light, *Cololabis saira* fishing vessels.

Class	The average of tons	Number of light pole	Lights per one pole (W)	Total (W)	Intervals of every pole (m)	Height of light (m)	The average of voltage (V)	Number of sampling ship
1	less than 20	3	500×10	15.000	6	2.8	130	8
2	more than 20	5	500×8	20.000	4	2.8	114	14
3	30	5	500×10	25.000	4	3	115	36
4	40	4	500×10	20.000	4	3	114	42
5	50	5	500× 9	22.500	4	3.2	111	16
6	70	6	500× 9	27.000	4	3.5	111	12
7	80	5	500× 7	17.500	5	3.5	106	7

The values of illumination calculated from Table 1 are shown in Table 2. Table 2 shows that *Cololabis saira* fishing is practised with illumination lux as in Table 2 in Japan.

Positions P_1 and P_2 in Fig. 1 indicate the places for which the strength of illumination at the central point was calculated in every run.

Table 2. Illumination from fishing vessels (lux).

Class	The average of tons	The depth of water (m.)					
		0		10		20	
	less than	P_1	632.8	P_1	31.2	P_1	4.3
1	20	P_2	308.9	P_2	24.6	P_2	3.8
	more than	P_1	673.4	P_1	38.3	P_1	5.4
2	20	P_2	353.4	P_2	34.0	P_2	5.0
		P_1	729.7	P_1	45.7	P_1	5.5
3	30	P_2	416.6	P_2	40.7	P_2	6.1
		P_1	729.7	P_1	45.7	P_1	6.6
4	40	P_2	416.6	P_2	40.7	P_2	6.1
		P_1	606.2	P_1	40.1	P_1	5.7
5	50	P_2	371.7	P_2	33.6	P_2	4.9
		P_1	722.8	P_1	39.5	P_1	6.1
6	70	P_2	357.7	P_2	37.9	P_2	5.9
		P_1	360.5	P_1	30.0	P_1	4.3
7	80	P_2	239.8	P_2	24.5	P_2	3.5

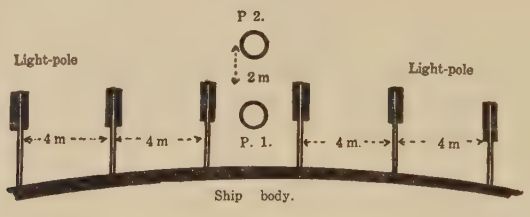

Fig. 1.

Arrangement of Experiment

These experiments were practised in a period from the 30th of October to the 6th of November in TOHOKU KAIKU. The fishing grounds were positioned at latitude 38°–13′N, longitude 142°–41′E and lat. 38°–27′N, long. 144°–18′E. Age of the moon was from 7.9th day to 13.9th day.

Illumination from the moon in this period was measured by an under water lux meter of the MURAYAMA type to be 0.3±0.01 lux.

Light of the training boat were used as follows.

Light pole No. 1.....white lamp 1,000 watt×2.

Light pole No. 2.....white lamp 1,000 watt×3 and 500 watt×1.

Light pole No. 3.....white lamp 1,000 watt×3 and 500 watt×2.

total being 9,500 watt.

Every light pole was fixed as in Fig. 2. Voltage was 100 volts. Illumination value was measured at the measurement point. Measured values are shown in Tables 3 and 4.

Table 3. Measured illumination values (lux).

Remarks: Light A (white lamp) 1000 w × 3; light B (white lamp) 1000 w × 3, 500 w × 1; light C (white lamp) 1000 w × 2, 500 w × 1; 9000 w, 100 V in total. Position in which the date observed, 38° 13′ N, 142° 48′ E; the age of moon 10.5 th.

Time	The depth of water (m)												N.B.
	0	0	1	2	3	4	5	7	10	12	15	20	
16 h-44 m	19.5	18	13	12	10.5	9.5	9.0	7.0	5.5	4.5	4.0	3.0	Time, sunset
17 h-32 m	110	100	80	65	45	30	25	20	10.2	7.0	5.0	2.3	Put on the light
21 h-27 m	145	110	95	85	70	50	40	20	14	9.5	6.0	3.4	〃
4 h-34 m	115	110	80	65	50	30	25	20	15	8.0	6.0	4.0	〃
5 h-15 m	128	125	85	70	55	45	35	25	15	8.0	6.0	4.5	Time, fish scattered
5 h-21 m	0.2	0.2	0.21	0.2	0.2	0.2							Put out the light

Table 4. Measured illumination values (lux).

Remarks: Position; lat. 38°—13′N, long. 142°—48′E; the time of measured from 22 h to 24 h: the age of moon 10.5 th.

Kind of the light	Height of the light (m)	The depth of water (m)												N. B.
		0	0	1	2	3	4	5	7	10	12	15	20	
500 watt × 2	1	190	150	120	75	55	30	20	16	5	3	1	0.7	White lamp
300 watt × 1	1	13	8	5.5	2.3	1.6	0.9	0.5	0	0	0	0	0	〃
500 watt × 1	1	63	45	34	20	11	8	5.5	3.0	1.8	1.0	0.6	0.2	〃
1.000 watt × 1	3	85	60	38	28	14	11	7	3	1.5	2	1.0	0	Day light lamp
500 watt × 1	3	79	59	50	30	19	12	9	5	2.3	1	0.5	0	〃

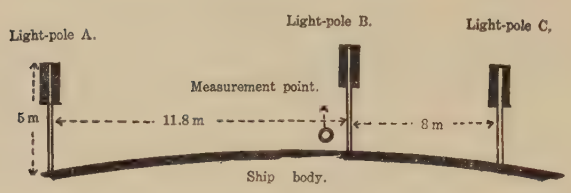

Fig. 2.

Result of experiment

Behavior of Cololabis saira towards light

Behavior was distinguished according to 3 types as follows.

1) *Cololabis saira* gathered into illuminated place in a jumping manner from darker place.

2) The fish gathered into the lighting zone in refloating motions upwards from dark and deeper water.

3) The fish assembled in a higher illumination place in irregular motions from dark places.

Group of *Cololabis saira* migrated into a place of highest illumination at the first time and swam there actively. Swimming layer of the fish was observed as to be one meter~4 meters in depth. Illumination of swimming layer was measured as 50~100 *lux*.

The fish dived for deeper water after a long time. The fish moved to dark place from light place and then the fish-finder recorded a fish group at 20–30 meters depth. This record means that the fish was swirling in 0.3~0.1 lux illumination zone. When the wind was strong, *Cololabis saira* gathered mostly to the light pole, which was positioned against the wind. The group of fish fell in confusion and scattered from the illuminated zone at break of day. At this time, the fish dived more deeply by degrees.

Daylight was observed as about 2 lux when the fish swam away from the light. *Cololabis saira* was found to be peculiar in choosing the wave-length of light. The fish was very fond of red system colours. The fish refloated willingly and speedily towards the light of red colour. The fish became animated suddenly on putting on a red light.

The fish could not refloat as directly towards light when cooler water was present above the layer in which the fish were swimming. (In the case such as shown in Table 5). Refloating of the fish was observed there after a long time. Fish appeared suddenly to the stronger light place from the lower illumination place when the high lux light was put on for swirling fish in less lightened place.

The fish refloated from 30 meters to 3 meters depth when the light was put out (according to a record of fish-finder). Relation between the *Cololabis saira's* body temperature and water temperature of swirling layer is as follows. Body temperature of *Cololabis saira* was measured to be 14.3°C. on the average and water temperature of swirling layer as 14.3°C.±1.6°C. (Testing fish were 60 individuals).

Table 5. Examples of lower temperature (°C) in upper water.

Depth of water (m)	0	5	10	20	30	40	50	60	70	80	90	100	110	200
Case I	15.95	15.9	15.9	15.9	15.9	15.4	15.4	15.4	12.0	11.0	9.0	8.4	8.0	4.97
Case II	15.3	15.4	15.6	16.2	16.2	16.4	15.3	14.1	12.1	11.6	11.0	10.2	10.2	
Fish body temperature	The average of fish-body temperature=14.3°C.													

Acknowledgement

The authors thank Mr. T. Kido, Y. Suzuki and the collaborator students for their assistance in these experiments.

Journal of the Tokyo University of Fisheries, Vol. 45, No. 2, 1959

STUDY ON THE DISPOSITION OF FISH TOWARDS LIGHT NO. 4. THE STRENGTH OF ILLUMINATION COMFORTABLE TO MACKEREL AND *TRACHUROUS JAPONICUS*

Yutaka IMAMURA

(Received Feb. 16, 1959)

Of what strength is the illumination that fishes are fond of?

The author has already pointed to the situation that it is generally very hard to reproduce a precise observation on phototaxis of fish as fishes behave themselves quite differently depending not only on the occasional strength of illumination but on the baits present. As he recently reported, in most cases of his experiments the carp (*Cyprinus carpio* LINNÉ) settled down in a place of lower illumination into a group formed in the dim place, while their individuals appeared in a lighter place alternatively from time to time. Throughout the observations continued for an hour in the preceding experiments, a largest group of carp was seen as a rule in a place at a lower illumination, while the group tended to become less crowded as the illumination was raised in strength. This time, the author experimented with *Mackerel* and *Trachurous japonicus* in a similar way to the preceding experiment in which carps were used as test fish. (Report No. 2).

Arrangement of Experiment

A concrete tank of the following size was used; 4.8 *m* in length, 1.5 *m* in breadth and 0.75 *m* in depth. The amount of water was adjusted to be 60 *cm*. The bottom surface of the tank was sectioned by white line into 27 sections equal in area, 50 cm^2. A reflecter lamp (100 volts 300 watts) was used as light source, which was set as shown in Fig. 1. The light was projected at an angle

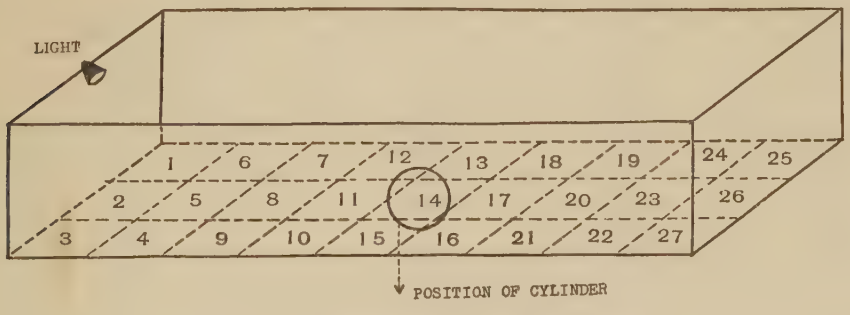

Fig. 1. Arrangement for the experiment.

of 45° to the water surface. The intensity of illumination was controlled by means of a slidax, while its measurement was done by an under-water lux meter of MURAYAMA type which was kept horizontal at the center of each section on the tank bottom.

A shadowed zone was prepared with appropriate boards in a randomly chosen place in the tank. As test fish, 50 individuals of *Trachurous japonicus* and 36 individuals of the *Mackerel* were provided in total. Average body length of the *Trachurous japonicus* was 16.5 *cm* and their mean weight was 18.5 *g*. Average body length of the *Mackerel* was 23 *cm*, their mean weight being 35 *g*.

Method of Experiment

1) A celluloid cylinder (50 *cm* in diameter, 70 *cm* in length) was set upright in an appointed place in the tank and the test fish were kept in this cylinder for ten minutes so as to be previously adapted to the dark.

2) The cylinder was then removed out of the tank with simultaneous putting on the light and setting the fish free.

3) Observing the behavior of fish, the pattern of fish distribution was recorded by counting the fish in each section at intervals of five minutes. Such observations were continued for one hour and repeated two times at each definite strength of illumination. Test fish were renewed for each run. Every 18 of *Mackerel* was used in every run, every 20 of *Trachurous japonicus* was used in

Table 1. Preliminaly experiment (*Mackerel*).

Section numbers	Number of fish observed (F)	Datum number of fish	Correction term (CT)	CT×2
1	0	8	8	16
2	0	8	8	16
3	0	8	8	16
4	7	8	1	2
5	17	8	− 9	−18
6	4	8	4	8
7	14	8	− 6	−12
8	45	8	−37	−74
9	14	8	− 6	−12
10	10	8	− 2	− 4
11	28	8	−20	−40
12	5	8	3	6
13	0	8	8	16
14	0	8	8	16
15	0	8	8	16
16	3	8	5	10
17	27	8	−19	−38
18	7	8	1	2
19	5	8	3	6
20	27	8	−19	−38
21	3	8	5	10
22	0	8	8	16
23	0	8	8	16
24	0	8	8	16
25	0	8	8	16
26	0	9	8	16
27	0	9	8	16
Σ	216	216	0	0
M	8	8	0	0

every experiment. The water temperature was kept at 18~18.5°C in all the runs. As these experiments have been performed in complete absence of any baits, the fish behavior observed may well be looked upon to be conducted purely by phototaxis of test fish.

A) **Preliminary Experiment**

It was proved in a preliminary experiment, which was carried out at zero *lux* illumination, that fish distribution is affected by the dimension of tank, the wall effect and the position in which the celluloid cylinder had been set. In this preliminary experiment the fish distributions at zero *lux* were inferred by conuting the fish in various sections by the help of momentary lightenings at intervals of five minutes (the duration of lightening was 5~7 seconds). The correction terms for the errors arising from the said factors were determined experimentally and are indicated in Table 1 and Table 2.

B) **Experiment 1.**

This experiment was run using an illumination being graded from section to section within a range of 2.35~39.5 *lux*. Test fish was *Mackerel*, every 18 individuals being used in duplicate.

Table 2. Preliminary experiment (*Trachurous japonicas*).

Section numbers	Number of fish observed (F)	Datum number of fish	Correction term (CT)	CT×2
1	0	9	9	18
2	0	9	9	18
3	0	9	9	18
4	0	9	9	18
5	5	9	4	8
6	2	9	7	14
7	2	9	7	14
8	30	9	−21	−42
9	3	9	6	12
10	6	9	3	6
11	15	9	−6	−12
12	0	9	9	18
13	1	9	8	16
14	22	9	−13	−26
15	5	9	4	8
16	7	9	2	4
17	58	9	−49	−98
18	2	9	7	14
19	3	9	6	12
20	59	9	−50	−100
21	10	9	−1	−2
22	3	9	6	12
23	7	9	2	4
24	0	9	9	18
25	0	9	9	18
26	0	9	9	18
27	0	9	9	18
Σ	240	≒240	≒0	≒0
M	≒9	≒9	≒0	≒0

C) **Experiment 2.**

An illumination graded within a range of 2.35~39.5 *lux* was used. Test fish was the *Trachurous japonicus*, every 20 individuals being used in duplicate.

* In these experiment, the author counted the number of fish in every section at the times given in the following lists (A & B) throughout the experiment and assumed that the number of fish indicates their disposition of preferring the illumination strength.

Result of Experiment

Experiment 1. (*Marckerel*)

Behavior of Mackerel:

Fish behavior observed in each experiment are given in the List A.

List A.

Times (minute)	*Out-lines of the behavior of Mackerel*
0 ↓ 5 ↓ 10 ↓ 15 ↓ 20 ↓ 30 ↓ 35 ↓ 40 ↓ 50 ↓ 60	The fish scattered at once, but they soon formed into two groups in the lighter place, every group migrated again from the lighter place to the lower illumination place. The main group settled down in a dim place and other group showed an elliptic motion in lighter place. The main group crowded into shadowed place, other group became somewhat diffused in the high illumination place, a few fish separated from this group and entered the main group. The main group settled down there. Then the main group was refloated to the middle-layer in a dim place and migrated calmly. A few fish swam all over. The main group separated into two groups and each group migrated along the side wall. The main group was resolved into individuals which swirled at random. Next time, the fish formed one group in the lighter place and moved toward a shadowed place slowly. The main group settled down in the dim place, a few individuals crowded in the lighter place. The main group moved for the high lux parts, but returned to the darker place at once. The main group swarmed calmly in the dim place and a few fish refloated to the upper-layer. The main group resolved into a few groups in the less illuminated place and migrated slowly along the wall. A main group was formed again in the lighter place and moved in a circular motion, returning to the shadowed place.

Response of a fish group to illumination:

In most cases, fish settled down in a place of lower illumination.

In a few cases, fish appeared in a lighter place. Fish crowded quietly in a shadowed place. But some individuals migrated activety to a higher illumination place. The mode of distribution of *Mackerel* is described in Table 3. The strength of illumination liked by fish is in an order of 2.6~32.5 *lux*. The data are shown in Tables 5 and 7. These data indicate that the most crowded parts took place up to 64.6% in total in a dim place. A crowded place of the next density was observed as far as 30% in total in a lighter zone.

Experiment 2. (*Trachurous japonicus*)

Behavior of Trachurous japonicus:

Fish behavior observed in each experiment is presented in List B.

Dependence of fish distribution upon illumination:

The results of examination are summarized in Table 4.

Table 6 and Table 8 have been obtained from the data in Table 2 and

Table 4. A largest assembly of fish was seen in illumination rank 1 (64.2%) and a fairly large one in rank 3 (24.9%). This result is quite similar to that for *Mackerel*.

Conclusion

When fish is left to freely move about under an illumination falling within a range of 2.35~39.5 *lux*, they react to light in the following manner:

1) A largest crowd of fish is formed in a place of least illumination (*Mackerel* and *Trachurous japonicus*).

As to their response to the illumination in the abovementioned range of strength *Mackerel* and *Trachurous japonicus* are not far different from Carp.

Table 3. Experiment (*Marckerel*).

Section number	Illumination		Number of sum fish			
	Lux	Rank	(F)	(CT)	(CV)	(CA)
1	32.0	3	0	16	16	59
2	39.5	3	40	16	56	99
3	33.0	3	7	16	23	66
4	28.0	3	23	2	25	68
5	35.5	3	96	−18	78	121
6	24.5	3	8	8	16	59
7	2.8	1	3	−12	−9	34
8	2.9	1	31	−74	*−43	0
9	2.9	1	8	−12	−4	39
10	5.1	2	0	−4	−4	39
11	5.5	2	0	−40	−40	3
12	4.8	2	0	6	6	49
13	2.8	1	0	16	16	59
14	3.0	1	0	16	16	59
15	2.9	1	0	16	16	59
16	2.5	1	0	10	10	53
17	2.8	1	0	−38	−38	5
18	2.5	1	0	2	2	45
19	2.5	1	0	6	6	49
20	2.5	1	45	−38	7	50
21	2.5	1	13	10	23	66
22	2.5	1	21	16	37	80
23	2.5	1	88	16	104	147
24	2.5	1	5	16	21	64
25	2.4	1	3	16	19	62
26	2.5	1	40	16	56	99
27	2.5	1	1	16	17	60
		Σ	432	0	432	1.593
		M	16	0	16	59

F: Number of fish observed in total.
CT: Correction term.
CV: Corrected value.
CA: Corrected values adjusted for comparison.

Table 5. Experiment (1) (*Mackerel*).

Illumination	Rank 1. (2.6 lux)			Rank 2. (5.1 lux)			Rank 3. (32.5 lux)		
	Lux	(F)	(CA)	Lux	(F)	(CA)	Lux	(F)	(CA)
	2.8	3	34	5.1	0	39	32	0	59
	2.9	31	0	5.5	0	3	39.5	40	99
	2.9	8	39	4.8	0	49	33	7	66
	2.8	0	59				28	23	68
	3.0	0	59				35.5	96	121
	2.9	0	59				24.5	8	59
	2.5	0	53						
	2.8	0	5						
	2.5	0	45						
	2.5	0	49						
	2.5	45	50						
	2.5	13	66						
	2.5	21	80						
	2.5	88	147						
	2.5	5	64						
	2.4	3	62						
	2.5	40	99						
	2.5	1	60						
	Σ	258	1.030		0	91		174	472
R.F. (%)		59.8	64.6		0	5.4		40.2	30.0
R.A. (%)		66			11			33	
A.F. (%)		14.3	57.2		0	5.0		9.7	26.2

F: Number of fish observed in total.
CA: Corrected values adjusted for comparison.
R.F.: Relative number of fish in a specified rank.
R.A.: Relative area of specified illumination.
A.F.: Average number of fish per section.

Table 7. Experiment 1. (*Mackerel*).

	Rank 1. (2.6 lux)		Rank 2. (5.1 lux)		Rank 3. (32.1 lux)	
	(F)	(CA)	(F)	(CA)	(F)	(CA)
R.F.	59.8%	64.6%	0%	5.4%	40.2%	30.0%
R.A.	66 %	66 %	11%	11 %	33 %	33 %
R.F./R.A.	0.90	0.98		0.49	1.65	0.90

F: Number of fish observed.
CA: Corrected values adjusted for comparison.
R.F.: Relative number of fish in a specified rank.
R.A.: Relative area of specified illumination.

List B.

Times (Minute)	*Outlines of the behavior of Trachurous japonicus*
0 *m*	Fish scattered at once and swam about at random. Fish formed a main group in the lighter place and then migrated for the dim place calmly. Following, the main group separated into two groups, a larger group settled down in the dim place and the other group refloated in the high illumination place.
10	The main group migrated to the lighter place, after a short times they returned to the dim place and settled down there. The main group swam irregulary, small group moved often for the lighter place and joined with the main group.
02	The main group moved for the shadowed place and was swirling there. A few fish rushed often to a lighter place and returned to the dim place. Two groups
30	settled down in the dim place. The smaller group joined to the main group in
40	the dim place. The main group refloated and was swirling in a circulary motion there. The main group remainned in dim place and a few fish were swirling
50	actively. The main group swam calmly in the dim place and continually repeated expansion and contraction by itself. The main group separated into
60	three groups and every group settled down in every shadowed place.

Table 4. Experiment (2) *Trachurous japonicus.*

Section number	Illumination		Number of sum fish			
	Lux	Rank	(F)	(CT)	(CV)	(CA)
1	32.0	3	0	18	18	79
2	39.5	3	22	18	40	101
3	33.0	3	2	18	20	81
4	28.0	3	8	18	26	87
5	35.5	3	39	8	47	108
6	24.5	3	1	14	15	76
7	2.8	1	3	14	17	78
8	2.9	1	37	−42	−5	56
9	2.9	1	10	12	22	83
10	5.1	2	7	6	13	74
11	5.5	2	27	−12	15	76
12	4.8	2	2	18	20	81
13	2.8	1	1	16	17	78
14	3.0	1	21	−26	−5	56
15	2.9	1	2	8	10	71
16	2.5	1	5	4	9	70
17	2.8	1	37	−98	*−61	0
18	2.5	1	3	14	17	78
19	2.5	1	7	12	19	80
20	2.5	1	108	−100	8	69
21	2.5	1	17	−2	15	76
22	2.5	1	17	12	29	90
23	2.5	1	94	4	98	159
24	2.5	1	2	18	20	81
25	2.5	1	0	18	18	79
26	2.5	1	6	18	24	85
27	2.5	1	2	18	20	81
		Σ	480	≒0	≒480	2.133
		M	≒18		≒18	79

F: Number of fish observed in total.
CT: Correction term.
CV: Corrected value.
CA: Corrected values adjusted for comparison.

Table 6. Experiment (2) (*Trachurous japonicus*).

Illumination	Rank 1. (2.6 lux)			Rank 2. (5.1 lux)			Rank 2. (32.1 lux)		
	Lux	(F)	(CA)	Lux	(F)	(CA)	Lux	(F)	(CA)
	2.8	3	78	5.1	7	74	32.0	0	79
	2.9	37	56	5.5	27	76	39.5	22	101
	2.9	10	83	4.8	2	81	33.0	2	81
	2.8	1	78				28.0	8	87
	2.0	21	56				35.5	39	108
	2.9	2	71				24.5	1	76
	2.5	5	70						
	2.8	37	0						
	2.5	3	78						
	2.5	7	80						
	2.5	108	69						
	2.5	17	76						
	2.5	17	90						
	2.5	94	159						
	2.5	2	81						
	2.4	0	79						
	2.5	6	85						
	2.5	2	81						
	Σ	372	1.370		36	231		72	532
R.F. (%)		77.5	64.2		7.5	10.9		15.0	24.9
R.A. (%)		66			11			33	
A.F. (%)		20.7	76.1		12	77.0		12	88.6

F: Number of fish observed in total.
CA: Corrected values adjusted for comparison.
R.F.: Relative number of fish in a specified rank.
R.A.: Relative area of specified illumination.
A.F.: Average number of fish per section.

Table 8. Experiment 2. (*Trachurous japonicus*).

	Rank 1.		Rank 3.		Rank 3.	
	(F)	(CA)	(F)	(CA)	(F)	(CA)
R.F.	77.5%	64.2%	7.5%	10.9%	15.0%	24.3%
R.A.	66 %	66 %	11 %	11 %	33 %	33 %
R.F./R.A.	1.17	0.97	0.68	0.99	0.45	0.75

F: Number of fish observed.
CA: Corrected values adjusted for comparion.
R.F.: Relative number of fish in a specified rank.
R.A.: Relative area of specified illumination.

It seems likely that the fishes would gather in a place of an illumination lower than 2.6 *lux*, if there were provided anywhere in their environment such a dark place as was actually present in the illumination range of 0.1～4.100 *lux* which was applied in the previous experiment to Carp. This situation may be emphasized of its reproduciblity from the observation that *Mackerel* and *Trachurous*

japonicus were always found to be fond of "a" dim place. Such a dim place, however, would have to be of an illumination a little higher than zero *lux*, as analogized from the experiment with Carp.

2) Fish-gathering value is determined as follows:

* R.F/R.A.....See Table 7 and 8.

3) Fish-gathering values are shown in Tables 7 and 8.

This table shows that the *Mackerel* crowded mainly to extent in rank 1 and *Trachurous japonicus* also assembled mainly in rank 1, while they did so only to a least extent in rank 3. Some individuals of *Mackerel* often went swiftly for a lighter place but *Trachurous japonicus* continued in the main to settled down in dim places for a long time. The observation points to that in the lighter zone *Mackerel* swam about more activety than *Trachurous japonicus*.

Acknowledgement

The author thanks Dr. H. Hirano cordinally for his kindness shown in making this manuscript presentable. The author thank the collaborator for their students assistance in these experiments.

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STUDY ON THE RESPONSE OF *TRACHUROUS JAPONICUS* TO AIR-BUBBLES

Yutaka Imamura and Michio Ogura

(Received Feb. 16, 1959)

In the angling of *Pagrosomus major* carried on in Chiba Prefecture, they use a fishing line with a lead (the sinker) is so designed as to evolve a small amount of air-bubble with the intention of drawing the fish near.

This mode of fishing is called Donburi Tsuri. In this connection it may be noticed that some reports from the Fisheries Laboratory, Busse-Bay in U.S.A. have informed the following matter. (In year 1957)

1) The herrings swam aside from the air-curtain.

2) The herrings became progressively confused while they kept shut up in a narrow place by means of the air curtain and then rushed across the air curtain to get loose as if they were being purseuned by an enemy. The author experimented on the behavior of *Tracchurous japonicus* to an air bubble appearing in the face of them.

Arrangement of Experimental Set

A wooden tank of the following size was used: 4 m in length, 0.6 m in breath and 0.6 m in depth. The amount of water was adjusted to be 50 cm in depth. This tank was sectioned into six divisions by thread and the experimental room was darkened and then two white lamps put on. Illumination of each section was represented by the lightness measured with a Murayama's under water *lux* meter kept horizontal at the center of each section on the tank bottom. An equipment for producing the air bubble was kept at the middle point of the border line separating section No. 4 from No. 5 shown in Fig. 1. To the nozzle of a can con-

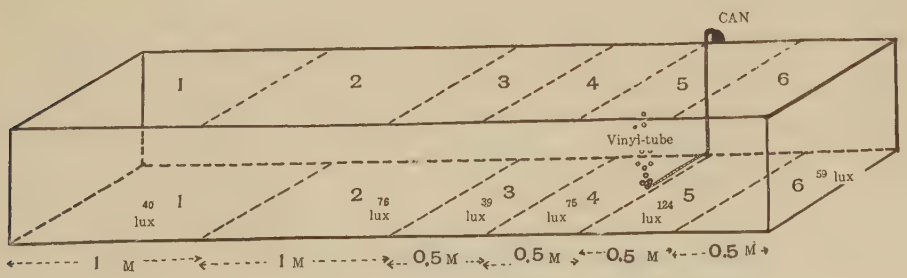

Fig. 1. Arrangement of the experiment.

taining CF_2Cl_2 gas at a pressure of 7 Atm was fitted a vinyl tube and air was discharged in bubbles into the watter from the end of the tube. As test fish, 85 individuals of *Trachurous japonicus* were avaiable in total. Every 10 of

them were used in every run. Their average body length was 16.5 cm and their mean weight was 35 g.

Method of Experiment

1) A celluloid cylinder (30 cm in diameter, 70 cm in length) was set upright at the center of No. 2 section and the test fish were put into this cylinder.

2) The cylinder was removed out of the tank, the fish being set free.

3) Observing the behavior of fish, the pattern of fish distribution was recorded by counting the fish in each section at intervals of one minute.

Such observations were continued for one hour or ten minutes and repeated some times under similar conditions. Test fish were renewed after each run. The water temperature was kept at 16°–18°C. in all run.

Experiment 1.

Observation was continued for a period of an hour. A) Preliminary experiment (without air bubble). B) Experiment proper (with air bubbles.) Illumination

Table 1. Experiment 1
Preliminary experiment (without air-bubble)

Time (min.) \ Section	Number of fish observed						Time (min.) \ Section	Number of fish observed					
	1	2	3	4	5	6		1	2	3	4	5	6
1	0	12	0	0	5	3	31	0	0	8	6	6	0
2	2	18	0	0	0	0	32	1	6	4	0	7	2
3	3	15	0	0	0	2	33	0	0	9	5	5	1
4	2	13	0	0	0	5	34	0	16	4	0	0	0
5	0	20	0	0	0	0	35	6	14	0	0	0	0
6	0	6	2	3	8	1	36	6	14	0	0	0	0
7	0	0	18	0	2	0	37	2	8	6	4	0	0
8	5	5	0	5	4	1	38	4	14	1	1	0	0
9	0	6	8	4	1	1	39	8	12	0	0	0	0
10	0	8	6	5	1	0	40	5	11	4	0	0	0
11	5	15	0	0	0	0	41	0	12	6	2	0	0
12	10	0	1	8	1	0	42	1	18	1	0	0	0
13	7	13	0	0	0	0	43	1	19	0	0	0	0
14	6	10	3	0	1	0	44	0	4	6	0	6	4
15	2	8	0	2	8	0	45	2	17	1	0	0	0
16	2	7	1	8	2	0	46	2	18	0	0	0	0
17	1	9	0	2	8	0	47	1	16	3	0	0	0
18	1	9	6	3	1	0	48	3	17	0	0	0	0
19	1	0	0	16	3	0	49	7	9	2	0	1	1
20	1	10	0	0	9	0	50	4	14	2	0	0	0
21	3	7	0	0	10	0	51	3	17	0	0	0	0
22	2	6	3	0	8	1	52	4	14	0	1	0	1
23	1	9	1	0	7	2	53	7	13	0	0	0	0
24	1	0	8	2	9	0	54	6	11	1	0	2	0
25	2	9	0	0	7	2	55	11	9	0	0	0	0
26	8	10	2	0	0	0	56	1	19	0	0	0	0
27	0	10	4	2	3	1	57	2	17	1	0	0	0
28	0	10	8	2	0	0	58	4	16	3	0	0	0
29	0	6	4	7	3	0	59	6	14	0	0	0	0
30	0	3	12	3	2	0	60	1	16	3	0	0	0
Total	65	254	87	72	103	19	Total	98	385	62	19	27	9

lux was kept constant for every run. Experiment was carried on in two runs. The results are shown in Tables 1 and 2.

Table 2. Experiment 1 (Experiment proper with air-bubble)

Time (min.) \ Section	Number of fish observed						Time (min.) \ Section	Number of fish observed					
	1	2	3	4	5	6		1	2	3	4	5	6
1	20	0	0	0	0	0	31	0	2	2	0	8	8
2	16	4	0	0	0	0	32	4	0	0	0	0	16
3	20	0	0	0	0	0	33	4	0	0	0	0	16
4	16	4	0	0	0	0	34	4	0	0	0	0	16
5	18	2	0	0	0	0	35	4	0	0	0	0	16
6	20	0	0	0	0	0	36	0	4	0	0	2	14
7	8	12	0	0	0	0	37	4	0	0	0	0	16
8	8	12	0	0	0	0	38	4	0	0	0	0	16
9	20	0	0	0	0	0	39	0	4	0	0	4	12
10	18	2	0	0	0	0	40	4	0	0	0	0	16
11	14	6	0	0	0	0	41	4	0	0	0	0	16
12	0	20	0	0	0	0	42	0	4	0	0	2	14
13	20	0	0	0	0	0	43	0	4	0	0	2	14
14	20	0	0	0	0	0	44	4	0	0	0	0	16
15	20	0	0	0	0	0	45	4	0	0	0	4	12
16	18	2	0	0	0	0	46	4	0	0	0	4	12
17	20	0	0	0	0	0	47	4	0	0	0	0	16
18	16	4	0	0	0	0	48	4	0	0	0	2	14
19	20	0	0	0	0	0	49	4	0	0	0	0	16
20	20	0	0	0	0	0	50	4	0	0	0	6	10
21	16	4	0	0	0	0	51	0	4	0	0	0	16
22	4	16	0	0	0	0	52	4	0	0	0	4	12
23	2	18	0	0	0	0	53	0	4	0	0	4	12
24	20	0	0	0	0	0	54	0	4	0	0	8	8
25	10	10	0	0	0	0	55	0	4	0	0	10	6
26	16	4	0	0	0	0	56	4	0	0	0	4	12
27	16	4	0	0	0	0	57	0	4	0	0	4	12
28	20	0	0	0	0	0	58	0	4	0	0	4	12
29	4	0	0	8	0	8	59	0	4	0	0	6	12
30	4	0	0	8	0	8	60	4	0	0	0	6	12
Total	444	124	0	16	0	16	Total	72	46	2	0	84	396

Experiment 2.

Behavior of fish when the volume of air bubble varied was observed. Results of experiments No. 1 shows that the fish is becomes familiar to air bubble. Influence of air bubble on fish weakened gradually with times; therefore every run in experiment No. 2 was continued only for ten minutes. Illumination was constant for every run.

A) *Preliminary experiment*

This experiment (without air bubbles) was repeated 2 times. The result is shown in Table 3.

B) *Experiment proper*

a) Volume of air bubble is 10, 11g or 13 g per 10 minutes (mean value 11g g/10 min) alternatively. The result is shown in Table 4.

b) Volume of air bubble was 15, 16 g or 20 g per 10 minutes. (mean value 17 g) alternatively. The result is shown in Table 5.

Table 3. Experiment 2. (Preliminary experiment....Without air bubble)

Time (min.) \ Section	Number of fish observed					
	1	2	3	4	5	6
(Total of 11 runs) 1	33	54	12	9	2	0
2	35	45	15	4	2	9
3	20	51	15	9	9	6
4	12	44	17	9	17	11
5	19	39	16	13	13	10
6	15	51	15	15	7	7
7	29	41	17	8	6	9
8	19	51	12	11	9	8
9	14	53	9	18	11	5
10	17	58	15	6	7	7
Total	213	487	143	102	83	72
Percentage of fish	20	45	13	9	7	6
Percentage of area	25	25	12.5	12.5	12.5	12.5
Datum number of fish	25	25	12.5	12.5	12.5	12·5
CT (Correction term)	5	−20	−0.5	3.5	5.5	6.5
CT×2	10	−40	−1	7	11	13
CT×3	15	−60	−1.5	10.5	16.5	19.5

Table 4. Experiment 2. (Experiment proper....With air bubbles 11 g).

Time (min.) \ Section	Number of fish observed					
	1	2	3	4	5	6
(Total of 3 runs) 1	20	10	0	0	0	0
2	8	22	0	0	0	0
3	10	20	0	0	0	0
4	10	20	0	0	0	0
5	8	22	0	0	0	0
6	8	22	0	0	0	0
7	10	20	0	0	0	0
8	7	23	0	0	0	0
9	8	22	0	0	0	0
10	14	16	0	0	0	0
Total	103	197	0	0	0	0
Percentage of area	25	25	12.5	12.5	12.5	12.5
Datum number of fish	75	75	37.5	37.5	37.5	37.5
CT×3	15	−60	−1.5	10.5	16.5	19.5
1st adjusted value	118	137	−1.5	10.5	16.5	19.5
2nd adjusted value	119.5	138.5	0	12	18	21
Percentage of fish	39	45	0	4	6	6

Table 5. Experiment 2. (Experiment proper....With air bubble, gas 17 g)

Time (min.) \ Section	Number of fish observed					
	1	2	3	4	5	6
(Total 3 runs) 1	30	0	0	0	0	0
2	28	2	0	0	0	0
3	30	0	0	0	0	0
4	26	4	0	0	0	0
5	28	2	0	0	0	0
6	29	1	0	0	0	0
7	22	8	0	0	0	0
8	20	10	0	0	0	0
9	22	8	0	0	0	0
10	28	2	0	0	0	0
Total	263	37	0	0	0	0
Percentage of area	25	25	12.5	12.5	12.5	12.5
Datum number of fish	75	75	37.5	37.5	37.5	37.5
CT (Correction term)	15	−60	−1.5	10.5	16.6	19.5
1st adjusted value	278	−23	−1.5	10.5	16.5	19.5
2nd adjusted value	301	0	21.5	33.5	39.5	42.5
Percentage of fish	72	0	5	7	8	8

Table 6. Experiment 2. (Experiment proper....With air bubble, gas 28 g)

Time (min.) \ Section	Number of fish observed					
	1	2	3	4	5	6
(Total of 3 runs) 1	29	1	0	0	0	0
2	30	0	0	0	0	0
3	29	1	0	0	0	0
4	28	2	0	0	0	0
5	27	3	0	0	0	0
6	28	2	0	0	0	0
7	28	2	0	0	0	0
8	27	3	0	0	0	0
9	29	1	0	0	0	0
10	29	1	0	0	0	0
Total	284	16	0	0	0	0
Percentage of area	25	25	12.5	12.5	12.5	12.5
Datum number of fish	75	75	37.5	37.5	37.5	37.5
CT (Correction term)	15	−60	−1.5	10.5	16.5	19.5
1st adjusted value	299	−44	−1.5	10.5	16.5	19.5
2nd adjusted value	343	0	42.5	54.5	60.5	63.5
Percentage of fish	60	0	7	10	11	12

c) Volume of air bubble: 25 and 30 g, per 10 minutes (mean value 28 g) alternatively. The result is shown in Table 6.

d) Volume of air bubble: 7 g. per 10 minutes. The result is shown in Table 7.

Table 7. Experiment 2 (Experiment proper....With air bubble, gas 7 g)

Time (min.) \ Section	Number of fish observed					
	1	2	3	4	5	6
(Total 2 runs) 1	17	2	1	0	0	0
2	18	2	0	0	0	0
3	18	2	0	0	0	0
4	18	2	0	0	0	0
5	17	3	0	0	0	0
6	16	4	0	0	0	0
7	14	6	0	0	0	0
8	17	3	0	0	0	0
9	17	2	1	0	0	0
10	17	3	0	0	0	0
Total	169	29	2	0	0	0
Percentage of area	25	25	12.5	12.5	12.5	12.5
Datum number of fish	50	50	25	25	25	25
CT (Correction term)	10	−40	−1	7	11	13
1st adjusted value	179	−11	1	7	11	13
2nd adjusted value	190	0	12	18	22	24
Percentage of fish	71	0	5	7	8	9

Experiment 3.

Behavior of fish was observed with variant illumination and with constant amount of air bubbles 12 g/10 min in every run. The experiment was carried on for ten minutes.

A) *With higher illumination*

a) Preliminary experiment (without air bubbles).

b) Experiment proper. (with air bubbles). The results of experiment are summarized in Table 8.

B) *With lower illumination*

a) Preliminary experiment (without air bubbles).

b) Experiment proper (with air bubbles). The results are shown in Table 9. The measured value of lux was given in Table 10.

Result of Experiment

Behavior of fish

1) *Trachurous japonicus* were watchful and afraid of the air bubbles in the early stage of these experiments and the fish escaped from the air bubbles.

Table 8. Experiment 3

Section / Time (min.)	Higher illumination											
	Without air bubbles						With air bubbles					
	1	2	3	4	5	6	1	2	3	4	5	6
1	2	1	0	0	3	4	2	5	0	0	3	0
2	1	4	3	1	1	0	0	2	3	4	1	0
3	3	6	0	1	0	0	0	3	3	2	2	0
4	2	8	0	0	0	0	0	4	3	2	1	0
5	2	7	0	1	0	0	0	1	6	3	0	0
6	9	1	0	0	0	0	1	7	1	1	0	0
7	8	2	0	0	0	0	0	3	1	3	2	1
8	9	1	0	0	0	0	0	3	3	4	0	0
9	8	2	0	0	0	0	1	6	2	1	0	0
10	5	5	0	0	0	0	1	3	2	2	1	1
Total	49	37	3	3	4	4	5	37	24	22	10	2
Percentage of area	25	25	12.5	12.5	12.5	12.5	25	25	12.5	12.5	12.5	12.5
Datum number of fish	25	25	12.5	12.5	12.5	12.5	25	25	12.5	12.5	12.5	12.5
Correction term	−24	−12	9.5	9.5	8.5	8.5	−24	−12	9.5	9.5	8.5	8.5
1st adjusted value							−19	25	33.5	31.5	18.5	10.5
2nd adjusted value							0	44	52.5	50.5	37.5	29.5
Percentage of fish							0	21	25	24	17	13

2) Fish became less watchful of air bubble by degrees with times.

3) After one hour, the fish were seen gathering around the air bubble. This disposition of fishes was observed in the later stages throughout the experiment.

* Analysis of fish distribution.

The author considers that the fish found in sections No. 4, No. 5, No. 3 and No. 6 have positively responded, the fish observed in No. 2 section are indifferent and such in No. 1 section have a dislike to air bubble.

Result of Experiment 1.

Table 2 clearly indicates that the behavior of fish shifts with the progress of time during the experiment.

Tables 11 and 12 have been derived from Tables 1 and 2 respectively. Table 11 tells that the fish gathering near the air bubble came to 21%, the fish remaining indifferent to air bubble to 6% and the fish escaping the bubble to 73% on the mean in a early half of one hour in the case of experiment 1.

Table 12 indicates that in a later half of one hour the fish gathering near the air bubble make 92%, the fish indifferent to the bubble 0% and the fish escaping 18%.

Table 9. Experiment 3

Section / Time (min.)	Lower illumination											
	Without air bubbles						With air bubbles					
	1	2	3	4	5	6	1	2	3	4	5	6
1	0	0	1	2	5	2	0	2	2	3	3	0
2	4	6	0	0	0	0	0	2	8	0	0	0
3	0	0	3	4	2	1	1	7	2	0	0	0
4	2	5	0	2	1	0	3	7	0	0	0	0
5	0	3	3	2	2	0	2	8	0	0	0	0
6	1	2	1	4	2	0	4	6	0	0	0	0
7	0	1	2	5	2	0	4	6	0	0	0	0
8	1	5	4	0	0	0	5	5	0	0	0	0
9	0	2	3	4	1	0	2	8	0	0	0	0
10	1	2	3	3	1	0	5	5	0	0	0	0
Total	9	26	20	26	16	3	26	56	12	3	3	0
Percentage of area	25	25	12.5	12.5	12.5	12.5	25	25	12.5	12.5	12.5	12.5
Datum number of fish	25	25	12.5	12.5	12.5	12.5	25	25	12.5	12.5	12.5	12.5
Correction term	16	−1	−7.5	−13.5	−3.5	9.5	16	−1	−7.5	−13.5	−3.5	9.5
1st adjusted value							42	55	4.5	−10.5	−0.5	9.5
2nd adjusted value							52.5	65.5	15	0	10	20
Percentage of fish							32	40	9	0	6	13

Table 10. Experiment 3 (The illumination was shown,in experiment 3.)

Illumination	Section					
	1	2	3	4	5	6
Higher (Lux)	39	57	79	150	100	37
Lower (〃)	0.2	0.4	0.5	1.0	0.6	0.3

Table 11. Early half of one hour. (Result of experiment 1)

Kind of experiment	Without air bubbles						With air bubbles					
Section	1	2	3	4	5	6	1	2	3	4	5	6
Percentage of area	25	25	12.5	12.5	12.5	12.5	25	25	12.5	12.5	12.5	12.5
Experimental data	65	254	87	72	103	19	444	124	0	16	0	16
Datum number of fish	150	150	75	75	75	75	150	150	75	75	75	75
Correction term	85	−104	−12	3	−28	56	85	−104	−12	3	−28	+56
1st adjusted value							529	20	−12	19	−28	72
2nd adjusted value							557	48	16	47	0	100
Percentage of fish							73	6	2	6	0	13

* Fish gathering....21%. Fish indifferent....6%. Fish escaping....73%.

Table 12. Later half of one hour. (Result of experiment 1)

Kind of experiment	Without air bubbles						With air bubbles					
Section	1	2	3	4	5	6	1	2	3	4	5	6
Percentage of area	25	25	12.5	12.5	12.5	12.5	25	25	12.5	12.5	12.5	12.5
Experimental data	98	385	62	19	27	9	72	46	2	0	84	396
Datum number of fish	150	150	75	75	75	75	150	150	75	75	75	75
Correction term	52	−235	13	56	48	66	52	−235	13	56	48	66
1st adjusted value							124	−189	15	56	132	462
2nd adjusted value							313	0	204	245	321	651
Percentage of fish							18	0	11	14	19	48

* Fish gathering....92%. Fish indifferent....0%. Fish escaping....18%.

Result of Experiment 2.

Table 13 has been derived from Tables 4, 5, 6 and 7. In these cases, volume of air bubble was graded within a range of 7 g∼28 g, but the volume influence of air bubble remained obscure.

Result of Experiment 3.

Table 14 has been derived from Tables 8 and 9. In higher illumination, fish showed a tendency of gathering near the air bubble. In lower illumination, fish tends to avoid the air bubble.

Table 13. Behavior of fish toward air-bubbles. (Result of experiment 2)

Gas (g)	Tendency		
	Gathered (%)	Indifferent (%)	Escaped (%)
10	22	32	46
28	46	0	54
17	25	5	70
7	33	2	66

Table 14. Behavior of fish toward air-bubbles. (Result of experiment 3)

Illumination	Tendency		
	Gathered %	Unrelated %	Avoided %
High	79	21	0
Lower	28	40	32

Conclusion

1) Angling of the *Pagrosomus major* is favored by the air bubble in respect of gathering fish in the following conditions, namely, as known, fine weather, the fishing ground with shallow water and clear water.

2) The author supposes from the present result that the effectiveness of air bubble or air curtain in chasing the herrings would attain an optimum in the following conditions, fishing ground in comparatively deep sea, a muddly water, and a relatively low illumination owing to cloudy weather.

Acknowledgement

The authors thanks the students for their assistance these experiments.

Journal of the Tokyo University of Fisheries, Vol. 45, No. 2, 1959

DETERMINATION OF TIME REQUIRED FOR FREEZING OF SKIP-JACK

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(Received Feb. 16, 1959)

Introduction

In Japan, frozen skip-jack (Katsuwonus pelamis (Linne)) is principal exposed-frozen-goods as material of canned tuna (light meat). Reduction of freezing-time of the fish and increasing of capacity of the freezer are important problems before us. Under such circumstances the authors induce a formula determining the time required for freezing of skip-jack, and carry out calculations to obtain some proper values.

Induction of Basic Formula

1. Freezing of Skip-Jack.

The fishes caught are chilled by sea-water cooled with ice on board a fishing vessels until the fishes are unloaded. Then, the fishes are washed, bled and frozen mainly in whole round type at a refrigerated warehouse on land.

2. Induction of Formula to Determine the Time Required for Freezing of Skip-Jack.

In general, the shape of whole round type fish like skip-jack will almost be regarded as a conical or a double conical body which is pasted together the same two conicals on their bases. Plank applied the theory of heat transmission for determination of the freezing-time of foods cylindrical in shape. According to the same method of Plank, the authors, regarding a fish as a conical or a double conical, induce a formula determining the time required for freezing of skip-jack.

In referring to Figs. 1 and 2, we let

B, H, L: total diameter of the base, total height and total length of the fish regarded as a conical or a double conical body in shape respectively m.

β: a half of vertical angle of the fish regarded as a conical or a double conical body in shape °.

γ_1, γ_2: specific weight of the fish in unfrozen and frozen state respectively kg/m³.

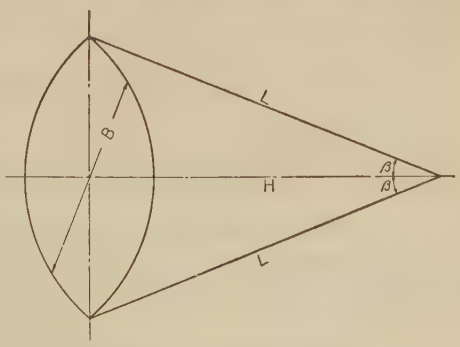

Fig. 1. Conical body.

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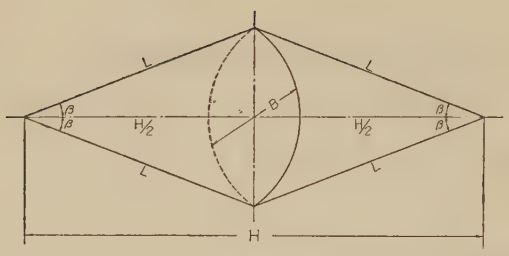

Fig. 2. Double conical body which is pasted together the same two comicals on their bases.

c_1, c_2: specific heat of the fish in unfrozen and frozen state respectively kcal/kg °C.

f: latent heat of freezing of the fish kcal/kg.

λ_1, λ_2: thermal conductivity of the fish in unfrozen and frozen state respectively kcal/mh °C.

θ_a, θ_b: initial and final temperature of the fish respectively °C.

θ_f: freezing point of the fish °C.

r: freezing percentage of the fish.

θ_e: temperature of the cooling medium °C.

α: heat transmission coefficient at the surface of the fish kcal/m²h °C.

Q: freezing-heat-quantity of the fish kcal.

q: freezing-heat-quantity of unit weight of the fish kcal/kg.

t: freezing-time of the fish h.

Then, if we assume that freezing of the fish proceeds uniformly from each surface to the center, and put B_0, H_0, L_0 for diameter of the base, height and length of unfrozen part of the fish respectively m, which is approximately regarded as a conical or a double conical body in shape.

a. In case the fish is regarded as a conical body in shape.

1) *in* freezing there are not any insulated surfaces in the fish and the fish radiates its heat through the whole surfaces.

The thickness of frozen part of the fish δ_0 m is at right angle to each surface and all equally given by

$$\delta_0=\frac{B-B_0}{2}\frac{\cos\beta}{1+\sin\beta}\ .$$

On the one hand, the freezing-heat-quantity dQ kcal taken away from the thin fish layer of which thickness is $d\delta_0$ m is given by

$$dQ=\{c_1(\theta_a-\theta_f)+c_1(1-r)(\theta_f-\theta_b)+fr+c_2r(\theta_f-\theta_b)\}\gamma_2\frac{\pi}{4}B_0^2(1+\mathrm{cosec}\,\beta)d\delta_0\ .$$

As $d\delta_0=-\frac{1}{2}\frac{\cos\beta}{1+\sin\beta}dB_0$ is derived from above relation, this equation is transformed into

$$dQ=-\{c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr\}\gamma_2\frac{\pi}{8}B_0^2(1+\mathrm{cosec}\,\beta)\frac{\cos\beta}{1+\sin\beta}dB_0\ .$$

On the other hand, this dQ kcal is expressed by heat transmission from the inner part of the fish to the cooling mediums through the whole surfaces of the fish during dt h as follows.

$$dQ=\frac{\frac{\pi}{4}(1+\mathrm{cosec}\,\beta)(\theta_f-\theta_e)dt}{\frac{\cos\beta}{2\lambda_2(1+\sin\beta)}\left(\frac{1}{B_0}-\frac{1}{B}\right)+\frac{1}{\alpha B^2}}\ .$$

Let each right term of these equations be equal and to carry out integration, and the freezing-time of the fish regarded as a conical in shape which has not any insulated surfaces and the fish radiates its heat through the whole surfaces can be obtained as follows.

$$t=\frac{\gamma_2}{24\lambda_2}B\frac{\cos\beta}{1+\sin\beta}\left(B\frac{\cos\beta}{1+\sin\beta}+\frac{4\lambda_2}{\alpha}\right)\frac{q}{\theta_f-\theta_e},$$

here

$$q=c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr.$$

Putting $B\frac{\cos\beta}{1+\sin\beta}=D$, the equation is transformed into

$$t=\frac{\gamma_2}{24\lambda_2}D\left(D+\frac{4\lambda_2}{\alpha}\right)\frac{q}{\theta_f-\theta_e},$$

here

$$q=c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr,$$

2) in freezing the base area of the fish is insulated and the fish radiates its heat through the side area.

We put $\sin\beta\to 0$ at the equation in case 1), following result to give the freezing-time of the fish of which base area is insulated and the fish radiates its heat through the side area can be obtained.

$$t=\frac{\gamma_2}{24\lambda_2}B\cos\beta\left(B\cos\beta+\frac{4\lambda_2}{\alpha}\right)\frac{q}{\theta_f-\theta_e},$$

here

$$q=c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr.$$

Putting $B\cos\beta=D$, the equation is transformed into

$$t=\frac{\gamma_1}{24\lambda_2}D\left(D+\frac{4\lambda_2}{\alpha}\right)\frac{q}{\theta_f-\theta_e},$$

here

$$q=c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr$$

3) in freezing the side area of the fish is insulated and the fish radiates its heat through the base area.

The thickness of frozen part of the fish δ_0 m is at right angle to the base area and given by

$$\delta_0=H-H_0 \quad \text{and} \quad \frac{B_0}{B}=\frac{H_0}{H}.$$

First, the freezing-heat-quantity dQ kcal taken away from the thin fish layer of which thickness is $d\delta_0$ m is given by

$$dQ=\{c_1(\theta_a-\theta_f)+c_1(1-r)(\theta_f-\theta_b)+fr+c_2r(\theta_f-\theta_b)\}\gamma_2\frac{\pi}{4}B_0^2d\delta_0.$$

As $d\delta_0=-dH_0$ and $B_0=(B/H)H_0$ are derived from above relations, this equation is transformed into

$$dQ=-\{c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr\}\gamma_2\frac{\pi}{4}\frac{B^2}{H^2}H_0^2dH_0.$$

Next, this dQ kcal is expressed by heat transmission from the inner part

of the fish to the cooling mediums through the base area of the fish during dt h as follows.

$$dQ=\frac{\frac{\pi}{4}(\theta_f-\theta_e)dt}{\frac{H}{\lambda_2 B^2}\frac{H-H_0}{H_0}+\frac{1}{\alpha B^2}}.$$

Let each right term of these equations be equal and to carry out integration, and the freezing-time of the fish conical in shape of which side area is insulated and the fish radiates its heat through the base area can be obtained as follows.

$$t=\frac{\gamma_2}{6\lambda_2}H\left(H+\frac{2\lambda_2}{\alpha}\right)\frac{q}{\theta_f-\theta_e}.$$

here

$$q=c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr.$$

Putting $H=D$, the equation is transformed into

$$t=\frac{\gamma_2}{6\lambda_2}D\left(D+\frac{2\lambda_2}{\alpha}\right)\frac{q}{\theta_f-\theta_e}.$$

here

$$q=c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr.$$

b. In case the fish is regarded as a double conical body which is pasted together the same two conicals on their bases in shape.

The thickness of frozen part of the fish δ_0 m is at right angle to each surface and all equally given by

$$\delta_0=\frac{B-B_0}{2}\cos\beta.$$

Again, on the one hand, the freezing-heat-quantity dQ kcal taken away from the thin fish layer of which thickness is $d\delta_0$ m is given by

$$dQ=\{c_1(\theta_a-\theta_f)+c_1(1-r)(\theta_f-\theta_b)+fr+c_2r(\theta_f-\theta_b)\}\gamma_2\frac{\pi}{2}B_0^2\operatorname{cosec}\beta d\delta_0.$$

As $d\delta_0=-(1/2)\cdot\cos\beta dB_0$ is derived from above equation, this equation is transformed into

$$dQ=-\{c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr\}\gamma_2\frac{\pi}{4}B_0^2\operatorname{cosec}\beta\cos\beta dB_0.$$

On the other hand, this dQ kcal is expressed by heat transmission from the inner part of the fish to the cooling mediums through the whole surfaces of the fish during dt h as follows.

$$dQ=\frac{\frac{\pi}{2}\operatorname{cosec}\beta(\theta_f-\theta_e)dt}{\frac{\cos\beta}{2\lambda_2}\left(\frac{1}{B_0}-\frac{1}{B}\right)+\frac{1}{\alpha B^2}}.$$

Let each right term of these equations be equal and to carry out integration, and the freezing-time of the fish regarded as a double conical which is

pasted together the same two conicals on their bases in shape and the fish radiates its heat through the whole surfaces can be obtained as follows.

$$t=\frac{r_2}{24\lambda_2}B\cos\beta\left(B\cos\beta+\frac{4\lambda_2}{\alpha}\right)\frac{q}{\theta_f-\theta_e},$$

here

$$q=c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr\ .$$

The solution of this case is the same in freezing the fish regarded as a conical in shape of which base area is insulated and radiates its heat through the side area. Then, putting $B\cos\beta=D$ also, the equation is transformed into

$$t=\frac{r_2}{24\lambda_2}D\left(D+\frac{4\lambda_2}{\alpha}\right)\frac{q}{\theta_f-\theta_e},$$

here

$$q=c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr\ .$$

c. In case the fish is frozen from its both sides opposited by direct contact with two cold metal plates.

This is a quite different freezing system compared to the above-mentioned one. The fish radiates its heat through two surfaces marked in its both sides opposited which is actually in contact with cold metal plates. Therefore these surfaces will be called an actual heat radiated surface. Then, if we assume that they are not only formed a rectangular form but also analogical to a sectional area at the central part of the fish, we can induce a formula determining the time required for freezing of the fish in this system.

In referring to Fig. 3 and above mentioned signs, we let

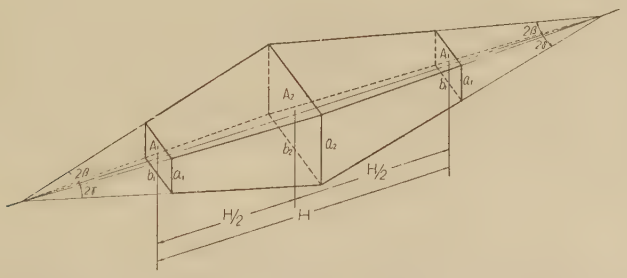

Fig. 3. Model in freezing of fish frozen from its both sides opposited by direct contact with two cold metal plates.

a, b, c: total body-width, total body-breadth and total body-length of the fish respectively m.

H: total thickness of the fish, which is a distance between the actual heat radiated surfaces opposited m.

a_1, b_1: height and width of the actual heat radiated surface of the fish which is approximately regarded as a rectangular form respectively m.
and put $a_1b_1=A_1$, here A_1: actual heat radiated surface area of the fish m^2.

a_2, b_2: height and width of the sectional area at the central part of the fish which is approximately regarded as a rectangular form respectively m.

and put $a_2b_2=A_2\fallingdotseq bc/2$, here A_2: sectional area at the central part of the fish m².

l_1, l_2: representative lengths of the actual heat radiated surface and the sectional area at the central part of the fish respectively m.

β, γ, β', γ': a half of vertical angle of the fish regarded as a rectangular cone body in shape °.

Then as a matter of course, the freezing of fish proceeds equally from both the actual heat radiated surfaces to the center, put a_0, b_0, H_0 for height, width and thickness which is a distance between both the actual heat radiated surfaces opposited of unfrozen part of the fish respectively m, the thickness of frozen part of the fish δ_0 m is at right angle to both the actual heat radiated surfaces opposited and given by

$$\delta_0=\frac{H-H_0}{2} .$$

On the one hand, the freezing-heat-quantity dQ kcal taken away from the thin fish layer of which thickness is $d\delta_0$ m is given by

$$dQ=\{c_1(\theta_a-\theta_f)+c_1(1-r)(\theta_f-\theta_b)+fr+c_2r(\theta_f-\theta_b)\}\gamma_2 2a_0b_0d\delta_0 .$$

As $d\delta_0=-(1/2)dH_0$ is derived from above relation, this equation is transformed into

$$dQ=-\{c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr\}\gamma_2a_0b_0dH_0 .$$

On the other hand, this dQ kcal is expressed by heat transmission from the inner part of the fish to the cooling mediums (exactly, to the two cold metal plates) through both the actual heat radiated surfaces opposited of the fish during dt h as follows.

$$dQ=\frac{2(\theta_f-\theta_e)dt}{\frac{1}{2\lambda_2}\frac{H-H_0}{\sqrt{a_1b_1a_0b_0}}+\frac{1}{\alpha a_1b_1}} .$$

Let each right term of these equations be equal and to carry out integration under taking into consideration following relations

$$\frac{b_1}{a_1}=\frac{b_0}{a_0}=\frac{b_2}{a_2} ,$$

$$a_0=\frac{2\tan\beta}{\cos\gamma'}(H-H_0)+a_1 ,$$

$$b_0=\frac{2\tan\gamma}{\cos\beta'}(H-H_0)+b_1 ,$$

and the freezing-time of the fish when it is frozen from its both sides opposited by direct contact with two cold metal plates can be obtained as follows.

$$t=\frac{\gamma_2}{24\lambda_2}H\left(\frac{a_1+2a_2}{a_1}H+\frac{a_1^2+a_1a_2+a_2^2}{a_1^2}\frac{4\lambda_2}{\alpha}\right)\frac{q}{\theta_f-\theta_e} ,$$

here

$$q=c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr .$$

In this equation, put into b_1, b_2 in place of a_1, a_2 respectively, and it gives the same result. Therefore a following transformation can be performed

$$\frac{a_1+2a_2}{a_1}=\frac{b_1+2b_2}{b_1}=\frac{l_1+2l_2}{l_1}=\frac{l_1l_2+2l_2^2}{l_1l_2}=\frac{\sqrt{A_1A_2}+2A_2}{\sqrt{A_1A_2}}\,.$$

$$\frac{a_1^2+a_1a_2+a_2^2}{a_1^2}=\frac{b_1^2+b_1b_2+b_2^2}{b_1^2}=\frac{l_1^2+l_1l_2+l_2^2}{l_1^2}=\frac{A_1+\sqrt{A_1A_2}+A_2}{A_1}\,.$$

Then, putting these relations into above equation and put $H=D$ also in this case, above equation is transformed into

$$t=\frac{r_2}{24\lambda_2}D\left(\frac{\sqrt{A_1A_2}+2A_2}{\sqrt{A_1A_2}}D+\frac{A_1+\sqrt{A_1A_2}+A_2}{A_1}\frac{4\lambda_2}{\alpha}\right)\frac{q}{\theta_f-\theta_e}$$

here

$$q=c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr\,.$$

As the actual heat radiated surface and the sectional area at the central part of the fish are assumed by analogical, the above equation can be always used in any form of these two areas so long as being analogical.

When the difference between A_2 and A_1 is not so large, we can carry out some abbreviation in this equation, it is simplified as follows.

$$t=\frac{r_2}{24\lambda_2}\frac{A_1+\sqrt{A_1A_2}+A_2}{A_1}D\left(D+\frac{4\lambda_2}{\alpha}\right)\frac{q}{\theta_f-\theta_e}\,.$$

here

$$q=c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr\,.$$

Through these solutions, we can obtain the following general formula to determine the time required for freezing of the fish regarded as a conical and a double conical body which is pasted together the same two conicals on their bases in shape like skip-jack.

$$t=\frac{r_2}{\mu\lambda_2}D\left(D+\frac{4\lambda_2}{\alpha}\right)\frac{q}{\theta_f-\theta_e}\,.$$

here

$$q=c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr\,.$$

freezing percentage of the fish r is approximately given by

$$r=1-\frac{\theta_f}{\theta_b}\,.$$

and μ: constant, D: mean (equivalent) diameter of the fish m.

The value of D changes relating to the value of μ as follows,

in freezing there are not any insulated surfaces in the fish regarded as a conical body in shape and the fish radiates its heat through the whole surfaces

$$\mu=24\,,\qquad D=B\frac{\cos\beta}{1+\sin\beta}\,.$$

in freezing not only the fish regarded as a conical in shape of which base area is insulated and radiates its heat through the side area, but also the fish regarded as a double conical in shape and radiates its heat through the whole surfaces

$$\mu=24\,,\qquad D=B\cos\beta\,.$$

in freezing the fish regarded as a conical in shape of which side area is insulated and radiates its heat through the base area

in $\mu=6$, $D=H$ and using $2\lambda_2/\alpha$ in place of $4\lambda_2/\alpha$.

freezing the fish is frozen from its both sides opposited by direct contact with two cold metal plates, and when the difference between A_2 and A_1 is not so large

$$\mu=\frac{24A_1}{A_1+\sqrt{A_1A_2}+A_2}, \qquad D=H.$$

In connection there-with, if we put W is weight of the fish, the freezing-heat-quantity taken away from the fish is given by in all cases

$$Q=Wq=W\{c_1(\theta_a-\theta_b)-(c_1-c_2)r(\theta_f-\theta_b)+fr\},$$

here

$$r=1-\frac{\theta_f}{\theta_b}.$$

Values of Same Physical Constants of Fish Muscle of Skip-Jack

A method to calculate the values of some physical constants of fish muscle has already been reported. General constituents of the fish muscle of skip-jack are about moisture content: 0.725, fat content: 0.025, solid matter content: 0.25 (protein 0.24+ash 0.01). Therefore the values of some physical constants of the fish muscle of skip-jack can be obtainable as follows.

specific weight of the fish in unfrozen state	$\gamma_1=1080$ kg/m^3.
specific weight of the fish in frozen state	$\gamma_2=1020$ kg/m^3.
specific heat of the fish in unfrozen state	$c_1=0.81$ kcal/kg °C.
specific heat of the fish in frozen state	$c_2=0.45$ kcal/kg °C.
latent heat of freezing of the fish	$f=58$ kcal/kg
thermal conductivity of the fish in unfrozen state	$\lambda_1=0.38$ kcal/mh°C.
thermal conductivity of the fish in frozen state	$\lambda_2=1.29$ kcal/mh°C.

Further, the freezing point of the fish muscle of skip-jack $\theta_f=-1$°C. and the freezing percentage of the fish muscle of skip-jack

$$r=1-\frac{-1}{\theta_b}.$$

Values of Some Shape Factors (Geometrical Constants) of Fish Body of Skip-Jack

In general, the shape of whole round fish like skip-jack is almost regarded as a conical or a double conical body which is pasted together the same two conicals on their bases in shape. Therefore following calculations can be carried out in whole round unfrozen fish like skip-jack.

First, if we let

a: body-width measured in the thickest part of the fish m.

b: body-breadth measured in the highest part of the fish m.

c: body-length measured from a snout to a jointed part of tail of the fish m.

we will be able to define following three linear shape factors.

$$\frac{c}{a}=k_1 \qquad \therefore \quad a=\frac{c}{k_1},$$

$$\frac{c}{b}=k_2 \qquad \therefore \quad b=\frac{c}{k_2},$$

$$\frac{b}{a}=k_3, \qquad \text{while as} \qquad a=\frac{c}{k_1}, \quad b=\frac{c}{k_2}, \quad k_3=\frac{k_1}{k_2}.$$

These values are all approximately considered as a constants in some definite kind of fish for instance skip-jack. Herewith it will be called that k_1: linear shape factor at body-length to body-width of the fish, k_2: linear shape factor at body-length to body-breadth of the fish and k_3: linear shape factor at body-breadth to body-width of the fish. Then, if we let

A: surface area of the fish m^2

V: volume of the fish m^3

we can approximately express these values as follows.

$$A=\frac{\pi}{k_4}\frac{a+b}{2}c,$$

$$V=\frac{\pi}{k_5}abc.$$

Therewith, it will be also called that k_4: shape factor at surface area of the fish and k_5: shape factor at volume of the fish.

As for whole round skip-jack, these values are approximately given by

$$k_1\fallingdotseq 5, \quad k_2\fallingdotseq 4 \quad \text{and so} \quad k_3=\frac{5}{4}.$$

$$k_4=\frac{6.5}{6}, \quad k_5=6.5.$$

Therefore, following relations can be obtainable.

$$a=\frac{c}{5}, \quad b=\frac{c}{4} \quad \text{and so} \quad b=\frac{5}{4}a.$$

$$A=\frac{\pi}{6.5/6}\frac{a+b}{2}c,$$

$$V=\frac{\pi}{6.5}abc,$$

and approximately

$$k_4\fallingdotseq\frac{k_5}{6}.$$

Next, if we let

W: body-weight of the fish kg

γ: specific weight of the fish in unfrozen state kg/m^3, and $\gamma=W/V$

we can obtain following other relations as for surface area and volume of the fish.

$$A=\frac{\pi}{k_4}\frac{a+b}{2}c=\frac{k_1+k_2}{2k_1k_2k_4}\pi c^2=\frac{\pi}{k_A}c^2,$$

$$V=\frac{\pi}{k_5}abc=\frac{\pi}{k_1k_2k_5}c^3=\frac{\pi}{k_V}c^3$$

Here, both k_A and k_V are also shape factor at surface area and volume of the fish respectively, and

$$k_A=\frac{2k_1k_2k_4}{k_1+k_2}\ , \qquad k_V=k_1k_2k_5\ .$$

If we use the values of each shape factor in whole round skip-jack, following values and relations can be obtainable.

$$k_A=\frac{130}{27}\ , \qquad k_V=130$$

therefore

$$A=\frac{27}{130}\pi c^2\ , \qquad V=\frac{\pi}{130}c^3\ .$$

Then, we transform $V=(\pi/130)c^3$ and $\gamma=W/V$ into

$$c=\sqrt[3]{\frac{130}{\pi}V} \quad \text{and} \quad V=\frac{W}{\gamma}\ .$$

Putting this V into c

$$c=\sqrt[3]{\frac{130}{\pi}\frac{W}{\gamma}}$$

and in skip-jack of unfrozen state

$$\gamma=\gamma_1=1080\ \text{kg/m}^3. \quad \text{and} \quad \pi=3.14\ ,$$

therefore

$$c=0.34\sqrt[3]{W} \quad \text{and} \quad b=\frac{c}{4}\ , \quad a=\frac{c}{5}\ ,$$

can be obtainable.

In connection with these relations, a sectional area of the fish is not a circle having diameter B m. but an ellipse having minor axis a m. and major axis b m. In formulas determining the time required for freezing of the fish induced previously, a sectional area of the fish is considered as a circle. Therefore, we must prefer some mean diameter B m. in place of body-width a m. and bodybreadth b m. In this case, we can take into consideration following three kinds of mean diameter.

by using arithmetic mean $B=(a+b)/2$

by using geometric mean $B=\sqrt{ab}$

by using harmonical mean $B=2ab/(a+b)$

Here, we use the harmonical mean $B=2ab/(a+b)$.

When the whole round type fish is regarded as a double conical body which is pasted together the same two conicals on their bases in shape

$$\tan\beta=\frac{B/2}{H/2}=\frac{B}{H} \quad \text{and} \quad B=\frac{2ab}{a+b}=\frac{2c}{k_1+k_2}\ , \quad H=c\ ,$$

therefore

$$\frac{B}{H}=\frac{2}{k_1+k_2} \qquad \therefore \ \tan\beta=\frac{2c}{k_1+k_2}\ ,$$

are induced. Putting $k_1=5$, $k_2=4$ as values of skip-jack into this relation, we can obtain some values in skip-jack as follows.

$$\tan\beta=0.222 \qquad \therefore\ \beta=\tan^{-1}0.222=12°30',$$
$$\therefore\ \sin\beta=\sin 12°30'=0.216,$$
$$\text{and}\quad \cos\beta=\cos 12°30'=0.976$$

therefore

$$\frac{\cos\beta}{1+\sin\beta}=\frac{0.976}{1+0.216}=0.803\fallingdotseq 0.8 \quad \text{and} \quad \cos\beta=0.976\fallingdotseq 1\ .$$

Therewith, mean (equivalent) diameter D m in whole round skip-jack is expressed as follows.

$$D=B\frac{\cos\beta}{1+\sin\beta}=\frac{2ab}{a+b}\frac{\cos\beta}{1+\sin\beta}\fallingdotseq 0.8\frac{2ab}{a+b}\ ,$$

$$D=B\cos\beta=\frac{2ab}{a+b}\cos\beta\fallingdotseq\frac{2ab}{a+b}\ .$$

Comparison of Experimental Results and Calculated Values of Freezing-Time of Skip-Jack

On the one hand, the freezing-curves of skip-jack were obtained experimentally by many freezing systems, on the other, the calculated values of freezing-time of skip-jack were obtained by using above formulas and some physical and geometrical constants. The freezing-curves are illustrated in Figs. 4~10.

1. Still Air-Sharp Type Freezing of Many Styles of Fish.

In Fig. 4, the fishes were treated in following four ways before freezing.

a. Whole round type i.e., uncut
b. Semi-dressed type i.e., eviscerated
c. Dressed type i.e., beheaded but ungutted in this case, so this is not dressed type under exact meaning.
d. Filleted type i.e., beheaded and gutted but uncut in this case, so this is not filleted type under exact meaning, but this is rather to be called dressed type.

Then, the fishes were put on a metal sheet over the cooling coils with ammonia evaporating within them and frozen under a still air; therefore, cooling mediums were both a cold still air and an evaporating ammonia through the pipe coils, and the fishes radiated their heat through the whole surfaces. In calculation, however, we took into consideration that cooling medium was a cold still air only. This was a still air-sharp type freezing. So, $\mu=24$ and $\alpha=10+4v$ here v: air velocity m/sec therefore $\alpha=10+4\times 0=10$ were used in all calculations.

a. In freezing of round type fish.

The freezing-curve obtained from the experiment shows that the raw whole rounded type fish having approximately a conical shape of $a\times b\times c=0.076\times 0.104\times 0.401$ m. in size, $W=1.850$ kg. in weight and initial temperature of the fish $\theta_a=+14.3$°C. was frozen at final temperature of the fish $\theta_b=-15.0$°C. (freezing

percentage of the fish $r=0.9333$) for time $t=15$ h. under average temperature of a cold still air $\theta_e=-10.0$°C. While the freezing-time of the fish t obtained from the calculation is 14.26 h under mean diameter of the fish $D=2ab/(a+b)=0.089$ m.

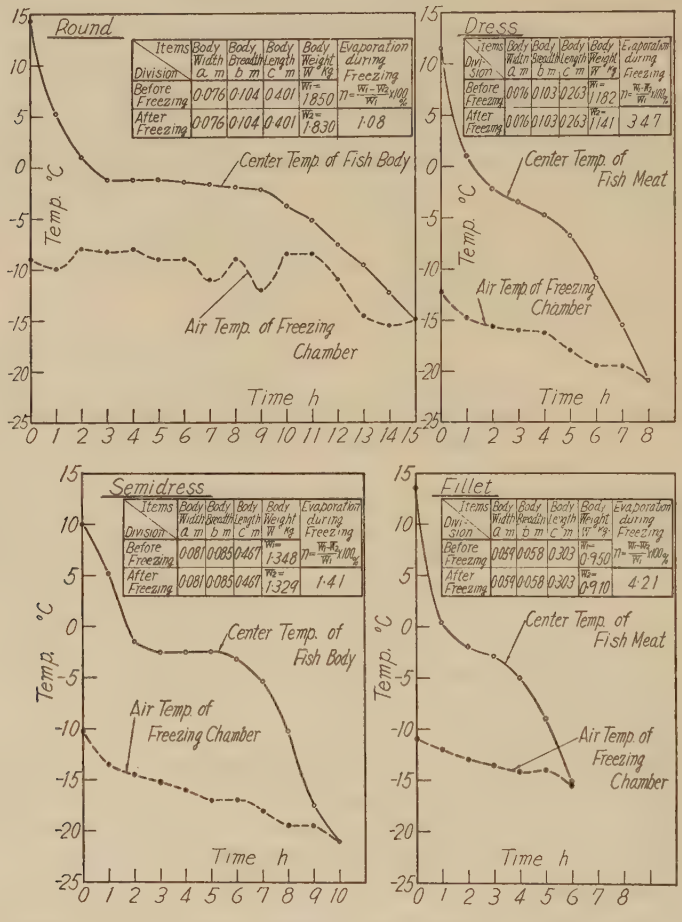

Fig. 4. Freezing-curves by still air-sharp type freezing of many styles of skip-jack.

b. In freezing of semi-dressed type fish.

The freezing-curve obtained from the experiment shows that the raw semi-dressed type fish having approximately a conical shape of $a\times b\times c=0.081\times 0.085\times 0.467$ m. in size, $W=1.348$ kg. in weight and initial temperature of the fish $\theta_i=+10.0$°C. was frozen at final temperature of the fish $\theta_b=-21.0$°C. (freezing percentage of the fish $r=0.9524$) for time $t=10$ h. under average temperature of a cold still air $\theta_e=-15.0$°C. While the freezing-time of the fish t obtained from the calculation is 8.61 h. under mean diameter of the fish $D=2ab/(a+b)=$ 0.083 m. In this case, as the original raw fish (the raw whole round type fish before evisceration) had had approximately a conical shape of $a\times b\times c=0.081\times 0.115\times 0.467$ m in size and $W=1.675$ kg in weight, the body-breadth of semi-dressed

type fish after evisceration has become $(0.085/0.115)\times100=73.91\%$ of that of original whole round type fish as well as the body-weight of semi-dressed type fish after evisceration has become $(1.348/1.675)\times100=80.48\%$ of that of original whole round type fish.

c. In freezing of dressed type fish.

The freezing-curve obtained from the experiment shows that the raw dressed type fish having approximately a conical shape of $a\times b\times c=0.076\times 0.103\times 0.263$ m in size, $W=1.182$ kg in weight and initial temperature of the fish $\theta_a=+11.5$°C. was frozen at final temperature of the fish $\theta_b=-21.0$°C. (freezing percentage of the fish $r=0.9524$) for time $t=8$ h under average temperature of a cold still air $\theta_e=-17.0$°C. While the freezing-time of the fish t obtained from the calculation is 6.33 h under mean diameter of the fish $D=0.8\cdot 2ab/(a+b)=0.070$ m. In this case, as the original raw fish (the raw whole round type fish before beheading) had had approximately a conical shape of $a\times b\times c=0.076\times 0.103\times 0.390$ m. in size and $W=1.470$ kg. in weight, the body-length of dressed type fish after beheading has become $(0.263/0.390)\times100=67.44\%$ of that of original whole round type fish as well as the body-weight of dressed type fish after beheading has become $(1.182/1.470)\times100=80.41\%$ of that of original whole round type fish.

d. In freezing of filleted type fish.

The freezing-curve obtained from the experiment shows that the raw dressed type fish having approximately a conical shape of $a\times b\times c=0.059\times 0.058\times 0.303$ m. in size, $W=0.950$ kg. in weight and initial temperature of the fish $\theta_a=+13.5$°C. was frozen at final temperature of the fish $\theta_b=-15.5$°C. (freezing percentage of the fish $r=0.9355$) for time $t=6$ h. under average temperature of a cold still air $\theta_e=-14.0$°C. While the freezing-time of the fish t obtained from the calculation is 4.79 h. under mean diameter of the fish $D=0.8\{2ab/(a+b)\}=0.046$ m. In this case, as the original raw fish (the raw whole round type fish before beheading and gutting) had had approximately a conical shape of $a\times b\times c=0.059\times 0.101\times 0.378$ m. in size and $W=1.350$ kg. in weight, the body-breadth and the body-length of filleted type fish after beheading and gutting have become $(0.058/0.101)\times100=57.43\%$ and $0.303/0.378\times100=80.16\%$ of that of original whole round type fish respectively as well as the body-weight of filleted type fish after beheading and gutting has become $(0.950/1.350)\times100=70.37\%$ of that of original whole round type fish.

2. Air-Blast Type Freezing.

In Fig. 5, the whole round fishes were put on a wooden plate over the cooling coils with ammonia evaporating within them and frozen. A surrounding cold air of the fishes was not only cooled by the cooling coils but forced circulated by a plate fan. Therefore cooling medium was a cold moving air and the fishes radiated their heat through the whole surfaces. This was an air-blast type freezing. So, $\mu=24$, $D=2ab/(a+b)$ and $\alpha=6+4v$ here v: air velocity m/sec were used in all calculations.

a. In freezing under air velocity $v=0$ m/sec.

The freezing-curve obtained from the experiment shows that the raw

whole round fish having approximately a conical shape of $a \times b \times c = 0.060 \times 0.090 \times 0.343$ m. in size, $W = 0.895$ kg. in weight and initial temperature of the fish $\theta_a = +15.0°C$. was frozen at final temperature of the fish $\theta_b = -19.0°C$. (freezing percentage of the fish $r = 0.9474$) for time $t = 14$ h. under average temperature of a cold still air $\theta_e = -14.0°C$. While the freezing-time of the fish t obtained from the calculation is 13.00 h under mean diameter of the fish $D = 0.072$ m and heat transmission coefficient at the surface of the fish $\alpha = 6 + 4 \times 0 = 6$ kcal/m²h°C.

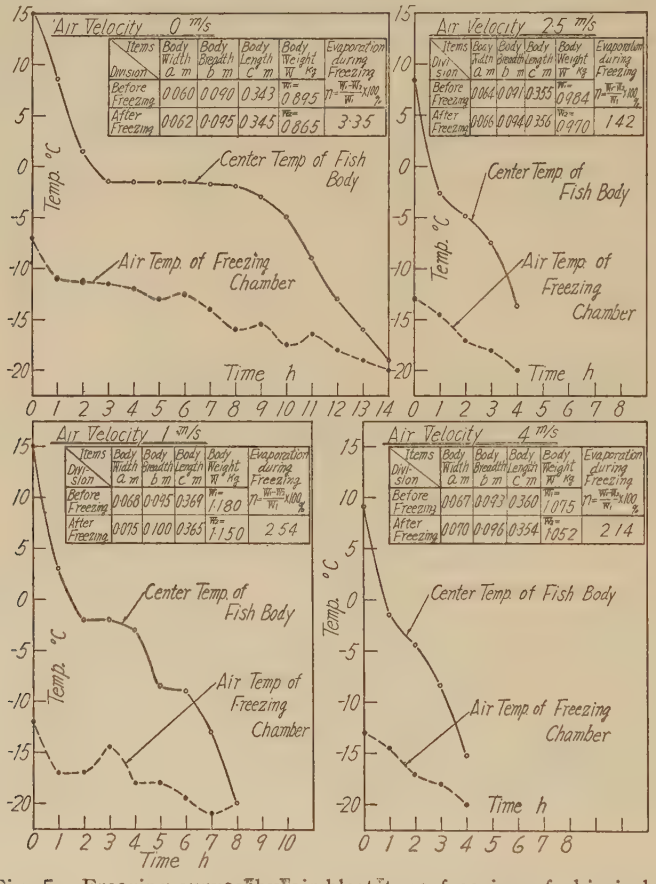

Fig. 5. Freezing-curves by air-blast type freezing of skip-jack.

b. In freezing under air velocity $v = 1$ m/sec.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a \times b \times c = 0.068 \times 0.095 \times 0.369$ m. in size, $W = 1.180$ kg. in weight and initial temperature of the fish $\theta_a = +15.0°C$. was frozen at final temperature of the fish $\theta_b = -20.0°C$. (freezing percentage of the fish $r = 0.95$) for time $t = 8$ h under average temperature of a cold moving air $\theta_e = -18.0°C$. While the freezing-time of the fish t obtained from the calculation is 7.03 h under mean diameter of the fish $D = 0.079$ m. and heat transmission coefficient at the surface of the fish $\alpha = 6 + 4 \times 1 = 10$ kcal/m²h°C.

c. In freezing under air velocity $v=2.5$ m/sec.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a\times b\times c=0.064\times 0.091\times 0.355$ m. in size, $W=0.984$ kg. in weight and initial temperature of the fish $\theta_a=+8.4$°C. was frozen at final temperature of the fish $\theta_b=-13.8$°C. (freezing percentage of the fish $r=0.9275$) for time $t=4$ h. under average temperature of a cold moving air $\theta_e=-17.0$°C. While the freezing-time of the fish t obtained from the calculation is 4.16 h under mean diameter of the fish $D=0.075$ m. and heat transmission coefficient at the surface of the fish $\alpha=6+4\times 2.5=16$ kcal/m²h°C.

d. In freezing under air velocity $v=4$ m/sec.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a\times b\times c=0.067\times 0.093\times 0.360$ m in size, $W=1.075$ kg. in weight and initial temperature of the fish $\theta_a=+9.0$°C. was frozen at final temperature of the fish $\theta_b=-15.2$°C. (freezing percentage of the fish $r=0.9342$) for time $t=4$ h. under average temperature of a cold moving air $\theta_e=-17.0$°C. While the freezing-time of the fish f obtained from the calculation is 3.47 h under mean diameter of the fish $D=0.078$ m. and heat transmission coefficient at the surface of the fish $\alpha=6+4\times 4=22$ kcal/m²h°C. In this case, as the original raw whole round fish was measured its volume V m³ by the Principle of Archimedes, each shape factor of the fish can be calculated. The raw fish have approximately a conical shape of $a\times b\times c=0.067\times 0.093\times 0.360$ m. in size, $V=0.00102$ m³. in volume and $W=1.075$ kg. in weight, so the values of each shape factor of the fish are determined as follows, the value of linear shape factor at body-length to body-width of the fish $k_1=c/a=0.360/0.067=5.37$, that of body-length to body-breadth of the fish $k_2=c/b=0.360/0.093=3.87$, that of body-breadth to body-width of the fish $k_3(=k_1/k_2)=b/a=0.093/0.067=1.39$, the value of shape factor at volume of the fish $k_5=(\pi/V)abc=(3.14/0.00102)\times 0.067\times 0.093\times 0.360=6.91$, by using this value the value of shape factor at surface area of the fish $k_4(=\pi/A\cdot(a+b)/2\cdot c$ here A: surface area m²) $\fallingdotseq k_5/6=1.15$ and the value of specific weight of the fish in unfrozen state $\gamma(=\gamma_1)=W/V=1.075/0.00102=1054$ kg/m³.

3. Still Air Type Freezing.

In Fig. 6, the whole round fish was hung from a ceiling at cold room and frozen by a still air, therefore cooling medium was a cold still air and the fish radiated its heat through the whole surfaces. This was a still air type freezing. So, $\mu=24$, $D=2ab/(a+b)$ and $\alpha=5+4v$ here v: air velocity m/sec therefore $\alpha=5+4\times 0=5$ were used in calculation.

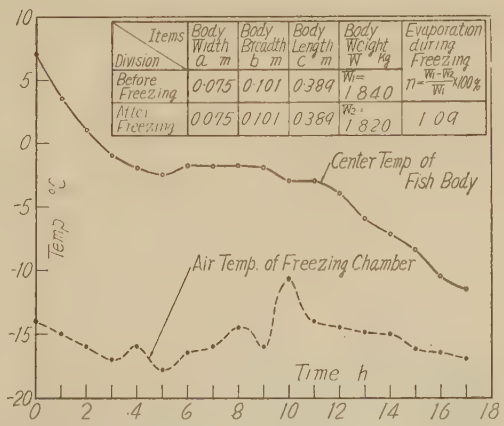

Fig. 6. Freezing-curve by still air type freezing of skip-jack.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a \times b \times c = 0.075 \times 0.101 \times 0.389$ m. in size, $W = 1.840$ kg. in weight and initial temperature of the fish $\theta_a = +7.0°C$. frozen at final temperature of the fish $\theta_b = -11.5°C$. (freezing percentage of the fish $r = 0.913$) for time $t = 17$ h. under average temperature of a cold still air $\theta_e = -15.0°C$. While the freezing-time of the fish t obtained from the calculation is 14.63 h. under mean diameter of the fish $D = 0.086$ m.

4. Semi-Air-Blast Type Freezing.

In Fig. 7, the whole round fishes were put on a metal sheet over the cooling coils with ammonia evaporating within them and frozen. A surrounding

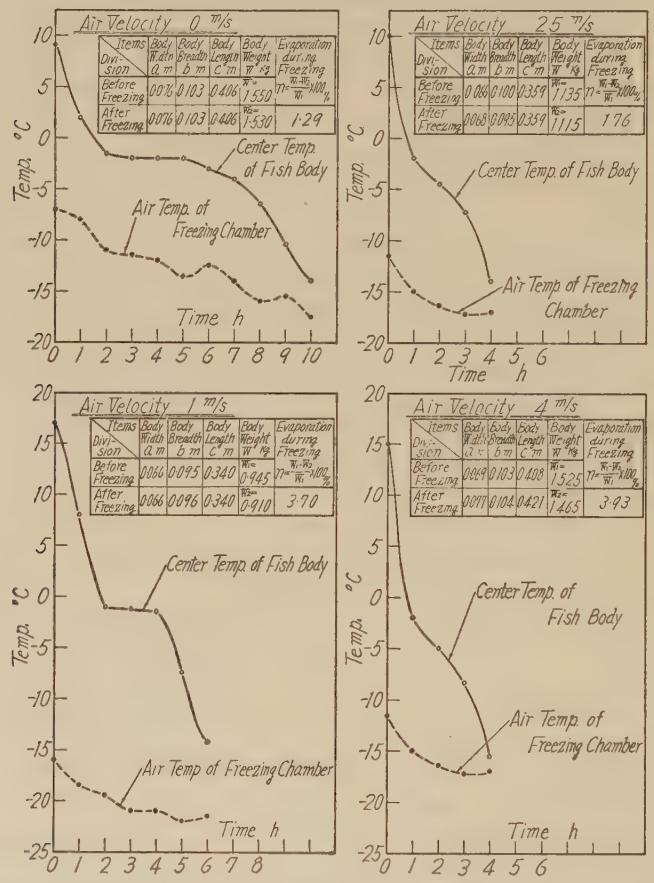
Air Velocity 0 m/s
Items | Division | Body Width a m | Body Breadth b m | Body Length c m | Body Weight W kg | Evaporation during Freezing
Before Freezing | 0.076 | 0.103 | 0.406 | W1= 1.550 | r1 = (W1−W2)/W1 ×100 %
After Freezing | 0.076 | 0.103 | 0.406 | W2= 1.530 | 1.29
Center Temp. of Fish Body
Air Temp. of Freezing Chamber
Temp. °C
Time h
Air Velocity 2.5 m/s
Items | Division | Body Width a m | Body Breadth b m | Body Length c m | Body Weight W kg | Evaporation during Freezing
Before Freezing | 0.046 | 0.100 | 0.359 | W1= 1.135 | r1 = (W1−W2)/W1 ×100 %
After Freezing | 0.068 | 0.095 | 0.359 | W2= 1.115 | 1.76
Center Temp. of Fish Body
Air Temp of Freezing Chamber
Temp. °C
Time h
Air Velocity 1 m/s
Items | Division | Body Width a m | Body Breadth b m | Body Length c m | Body Weight W kg | Evaporation during Freezing
Before Freezing | 0.066 | 0.095 | 0.340 | W1= 0.945 | r1 = (W1−W2)/W1 ×100 %
After Freezing | 0.066 | 0.096 | 0.340 | W2= 0.910 | 3.70
Center Temp. of Fish Body
Air Temp of Freezing Chamber
Temp. °C
Time h
Air Velocity 4 m/s
Items | Division | Body Width a m | Body Breadth b m | Body Length c m | Body Weight W kg | Evaporation during Freezing
Before Freezing | 0.069 | 0.103 | 0.408 | W1= 1.525 | r1 = (W1−W2)/W1 ×100 %
After Freezing | 0.077 | 0.104 | 0.421 | W2= 1.465 | 3.93
Center Temp. of Fish Body
Air Temp. of Freezing Chamber
Temp. °C
Time h

Fig. 7. Freezing-curve by semi-air-blast type freezing of skip-jack.

cold air of the fishes was not only cooled by the cooling coils but forced circulated by a plate fan. Therefore cooling mediums were both a cold moving air and an evaporating ammonia through the pipe coils, and the fishes radiated their heat through the whole surfaces. In calculation, however, we took into consideration

that cooling medium was a cold moving air only. This was a semi-air-blast type freezing. So, $\mu=24$, $D=2ab/(a+b)$ and $\alpha=10+4v$ here v: air velocity m/sec. were used in all calculations.

a. In freezing under air velocity $v=0$ m/sec.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a\times b\times c=0.076\times 0.103\times 0.406$ m. in size, $W=1.550$ kg. weight and initial temperature of the fish $\theta_a=+9.0°C$. was frozen at final temperature of the fish $\theta_b=-14.0°C$. (freezing percentage of the fish $r=0.9286$) for time $t=10$ h. under average temperature of a cold still air $\theta_e=-13.0°C$. While the freezing-time of the fish t obtained from the calculation is 9.83 h. under mean diameter of the fish $D=0.087$ m. and heat transmission coefficient at the surface of the fish $\alpha=10+4\times 0=10$ kcal/m²h°C.

b. In freezing under air velocity $v=1$ m/sec.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a\times b\times c=0.066\times 0.095\times 0.340$ m. in size, $W=0.945$ kg. in weight and initial temperature of the fish $\theta_a=+17.0°C$. was frozen at final temperature of the fish $\theta_b=-14.2°C$. (freezing percentage of the fish $r=0.9296$) for time $t=6$ h. under average temperature of a cold moving air $\theta_e=-20.0°C$. While the freezing-time of the fish t obtained from the calculation is 4.53 h under mean diameter of the fish $D=0.078$ m and heat transmission coefficient at the surface of the fish $\alpha=10+4\times 1=14$ kcal/m²h°C.

c. In freezing under air velocity $v=2.5$ m/sec.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a\times b\times c=0.066\times 0.100\times 0.359$ m. in size, $W=1.135$ kg. in weight and initial temperature of the fish $\theta_a=+10.0°C$. was frozen at final temperature $\theta_b=-14.0°C$. (freezing percentage of the fish $r=0.9286$) for time $t=4$ h. under average temperature of a cold moving air $\theta_e=-15.0°C$. While the freezing-time of the fish t obtained from the calculation is 4.40 h. under mean diameter of the fish $D=0.080$ m. and heat transmission coefficient at the surface of the fish $\alpha=10+4\times 2.5=20$ kcal/m²h°C.

d. In freezing under air velocity $v=4$ m/sec.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a\times b\times c=0.069\times 0.103\times 0.408$ m. in size, $W=1.525$ kg. in weight and initial temperature of the fish $\theta_a=+15.0°C$. was frozen at final temperature of the fish $\theta_b=-15.6°C$. (freezing percentage of the fish $r=0.9359$) for time $t=4$ h. under average temperature of a cold moving air $\theta_e=-15.0°C$. While the freezing-time of the fish t obtained from the calculation is 4.08 h. under mean diameter of the fish $D=0.083$ m. and heat transmission coefficient at the surface of the fish $\alpha=10+4\times 4=26$ kcal/m²h°C. In this case, as the original raw whole round fish was measured its volume V m³. by the Principle of Archimedes, each shape factor of the fish can be calculated. The raw fish have approximately a conical shape of $a\times b\times c=0.069\times 0.103\times 0.408$ m. in size, $V=0.00142$ m³. in volume and $W=1.525$ kg. in weight, so the values of each shape

factor of the fish are determined as follows, the value of linear shape factor at body-length to body-width of the fish $k_1=c/a=0.408/0.069=5.91$, that of body length to body-breadth of the fish $k_2=c/b=0.408/0.103=3.96$, that of body-breadth to body-width of the fish $k_3(=k_1/k_2)=b/a=0.103/0.069=1.49$, the value of shape factor at volume of the fish $k_5=(\pi/V)abc=(3.14/0.00142)\times 0.069\times 0.103\times 0.408=6.41$, by using this value the value of shape factor at surface area of the fish $k_4(=\overline{\pi/A\cdot(a+b)/2\cdot c}$ here A: surface area of the fish $m^2)\fallingdotseq k_5/6=1.07$ and the value of specific weight of the fish in unfrozen state $\gamma(=\gamma_1)=W/V=1.525/0.00142=1074\,kg/m^3$.

5. Forced Air-Sharp Type Freezing.

In Fig. 8, the whole round fish was put on a iron cover over the cooling coils (bound and tried them) with ammonia evaporating within them and frozen under a still air, therefore cooling mediums were both a cold still air and an evaporating ammonia through the pipe coils, and the fish radiated its heat through the whole surfaces. In calculation, however, we took into consideration that cooling medium was a cold still air only. This was a forced air-sharp type freezing. So, $\mu=24$, $D=2ab/(a+b)$ and $\alpha=12+4v$ here v: air velocity therefore $\alpha=12+4\times 0=12$ were used in calculation.

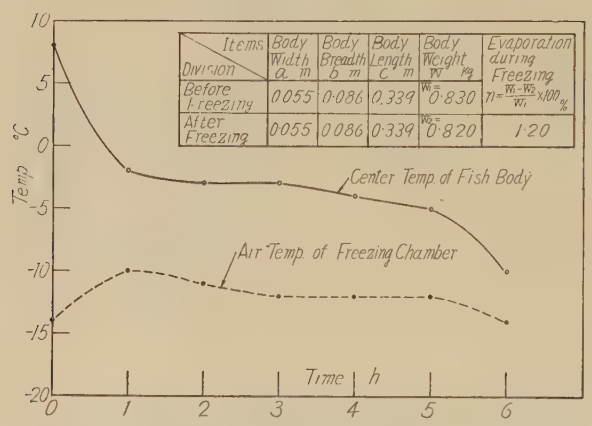

Fig. 8. Freezing-curve by forced air-sharp type freezing of skip-jack.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a\times b\times c=0.055\times 0.086\times 0.339$ m. in size, $W=0.830$ kg. in weight and initial temperature of the fish $\theta_a=+8.0°C$. was frozen at final temperature of the fish $\theta_b=-10.0°C$. (freezing percentage of the fish $r=0.9$) for time $t=6$ h. under average temperature of a cold still air $\theta_e=-13.0°C$. While the freezing-time of the fish t obtained from the calculation is 5.85 h under mean diameter of the fish $D=0.067$ m.

6. NaCl-Brine Immersion Type Freezing.

In Fig. 9, the whole round fishes were immersed in a cold NaCl-brine forced circulated by a centrifugal pump and frozen. Therefore cooling medium was a cold moving NaCl-brine, and the fishes radiated their heat through the whole surfaces. This was an NaCl-brine immersion type freezing. So, $\mu=24$, $D=2ab/(a+b)$ and $\alpha=30+50\sqrt{v}$ here v: NaCl-brine velocity m/sec were used in all calculations.

a. In freezing under NaCl-brine velocity $v=0$ m/sec.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a\times b\times c=0.062\times 0.084\times 0.320$ m.

in size, $W=1.040$ kg. in weight and initial temperature of the fish $\theta_a=+8.5°C$. was frozen at final temperature of the fish $\theta_b=-10.0°C$. (freezing percentage of the fish $r=0.9$) for time $t=6$ h. under average temperature of a cold still NaCl-brine $\theta_e=-8.0°C$. While the freezing-time of the fish t obtained from the calculation is 5.23 h. under mean diameter of the fish $D=0.071$ m. and heat transmission coefficient at the surface of the fish $\alpha=30+50\sqrt{0}=30+50\times 0=30$ kcal/m²h°C.

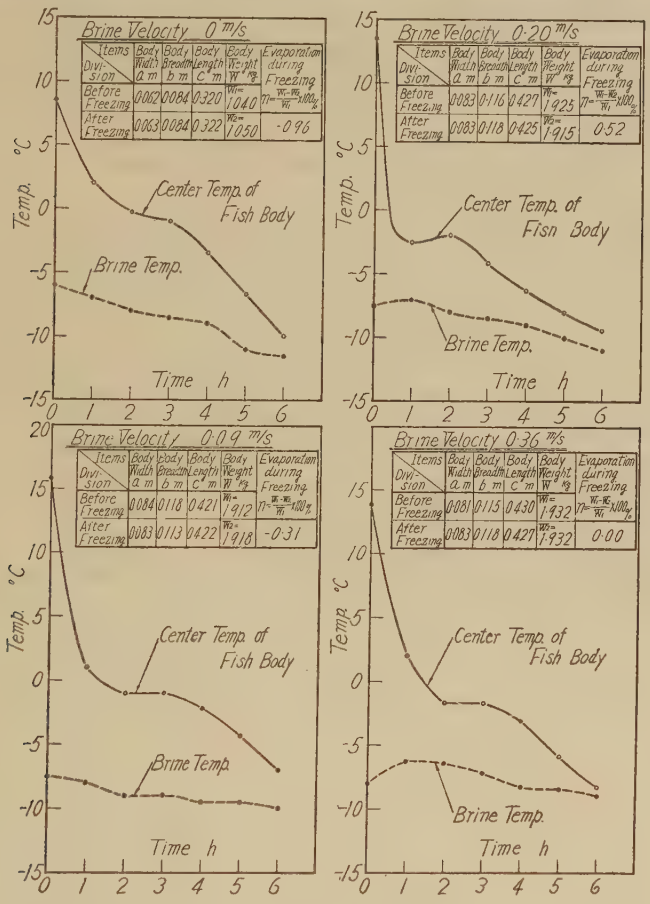
Brine Velocity 0 m/s
Items | Body Width a m | Body Breadth b m | Body Length c m | Body Weight W kg | Evaporation during Freezing
Division
Before Freezing | 0.082 | 0.084 | 0.320 | W1= 1.040 | π= (W1−W2)/W1 ×100 %
After Freezing | 0.063 | 0.084 | 0.322 | W2= 1.050 | -0.96
Brine Velocity 0.20 m/s
Before Freezing | 0.083 | 0.116 | 0.427 | W1= 1.925 | 0.52
After Freezing | 0.083 | 0.118 | 0.425 | W2= 1.915
Brine Velocity 0.09 m/s
Before Freezing | 0.084 | 0.118 | 0.421 | W1= 1.912 | -0.31
After Freezing | 0.083 | 0.113 | 0.422 | W2= 1.918
Brine Velocity 0.36 m/s
Before Freezing | 0.081 | 0.115 | 0.430 | W1= 1.932 | 0.00
After Freezing | 0.083 | 0.118 | 0.427 | W2= 1.932
Temp. °C
Time h
Center Temp. of Fish Body
Brine Temp.

Fig. 9. Freezing-curve by NaCl-brine immersion type freezing of skip-jack.

b. In freezing under Nacl-brine velocity $v=0.09$ m/sec.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a\times b\times c=0.084\times 0.118\times 0.421$ m. in size, $W=1.912$ kg. in weight and initial temperature of the fish $\theta_a=+15.8°C$. was frozen at final temperature of the fish $\theta_b=-7.0°C$. (freezing percentage of the fish $r=0.8571$) for time $t=6$ h. under average temperature of a cold moving NaCl-brine $\theta_e=-9.0°C$. While the freezing-time of the fish t obtained from the calculation is 5.71 h under mean diameter of the fish $D=0.098$ m and heat transmission coefficient at the surface of the fish $\alpha=30+50\sqrt{0.09}=30+50\times 0.3=45$ kcal/m²h°C.

c. In freezing under NaCl-brine velocity $v=0.20$ m/sec.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a\times b\times c=0.083\times 0.116\times 0.427$ m in size, $W=1.925$ kg. in weight and initial temperature of the fish $\theta_a=+13.5$°C. was frozen at final temperature of the fish $\theta_b=-9.4$°C. (freezing percentage of the fish $r=0.8936$) for time $t=6$ h. under average temperature of a cold moving NaCl-brine $\theta_e=-8.0$°C. While the freezing-time of the fish t obtained from the calculation is 6.04 h. under mean diameter of the fish $D=0.097$ m. and heat transmission coefficient at the surface of the fish $\alpha=30+50\sqrt{0.20}=30+50\times 0.45=52.5$ kcal/m²h°C.

d. In freezing under NaCl-brine velocity $v=0.36$ m/sec.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a\times b\times c=0.081\times 0.115\times 0.430$ m. in size, $W=1.932$ kg. in weight and initial temperature of the fish $\theta_a=+13.9$°C. was frozen at final temperature of the fish $\theta_b=-8.3$°C. (freezing percentage of the fish $r=0.8795$) for time $t=6$ h. under average temperature of a cold moving NaCl-brine $\theta_e=-8.0$°C. While the freezing-time of the fish t obtained from the calculation is 5.41 h under mean diameter of the fish $D=0.095$ m. and heat transmission coefficient at the surface of the fish $\alpha=30+50\sqrt{0.36}=30+50\times 0.6=60$ kcal/m²h°C.

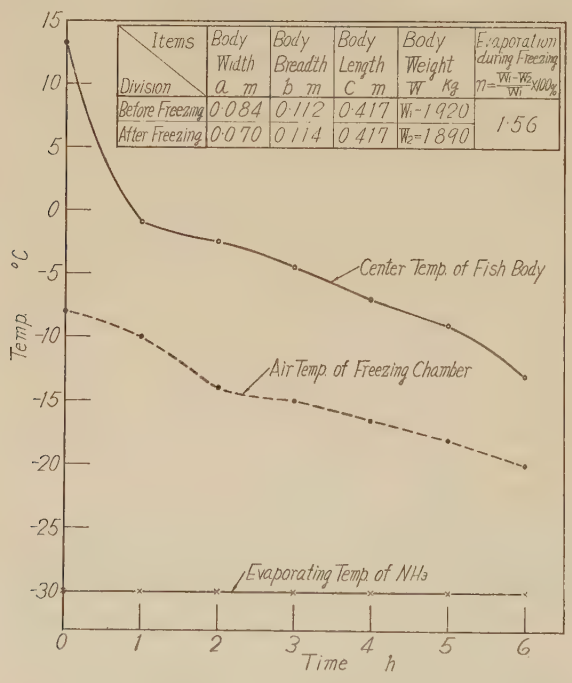

Fig. 10. Freezing-curve by double contact pressure type freezing of skip-jack.

7. Double Contact Pressure Type Freezing.

In Fig. 10, the whole round fish was inserted between the two cooling pipes with ammonia evaporating within them and frozen. The fish was cooled not only by direct contact with the cold pipe coils on its both sides, but also by a surrounding cold still air of the fish. Therefore cooling mediums were both an evaporating ammonia through the pipe coils and a cold still air, and the fish radiated its heat mainly through the two surfaces opposited being in contact with pipe coils. In calculation, however, we took into consideration that cooling medium was an evaporating ammonia through the pipe coils only. This was a double contact pressure type or so-called multi-plate type

freezing. But in Japan, such type freezer is not practically used in freezing of the whole round fish with a gut as skip-jack. This experiment was only carried out in order to obtain some comparative values against other types of freezer. So, $\mu=24$, $D=H$ and $\alpha=60$ were used in calculation.

The freezing-curve obtained from the experiment shows that the raw whole round fish having approximately a conical shape of $a\times b\times c=0.084\times 0.112\times 0.417$ m. in size, $W=1.920$ kg. in weight and initial temperature of the fish $\theta=+13.2$°C. was frozen at final temperature of the fish $\theta_b=-13.0$°C. (freezing percentage of the fish $r=0.9231$) for time $t=6$ h. under average temperature of an evaporating ammonia through the pipe coils $\theta_e=-30.0$°C. While the freezing-time of the fish t obtained from the calculation is 7.28 h under mean diameter of the fish during freezing $D=0.070$ m. (body-width of the fish not before freezing but after freezing and this is also considered an interval of the two pipe coils where just being inserted the sample), actual heat radiated surface area $A_1=0.12\times 0.03=0.0036$ m^2. (approximately rectangular form in shape marked at the surfaces of the fish after freezing) and a sectional area at the central part of the fish $A_2\fallingdotseq bc/2=(0.112\times 0.417)/2=0.0234$ m^2. As a difference of these values of A_1 and A_2 is not so small, the original formula in this case was used in calculation.

If all values of calculated freezing-time are compared to those obtained from the freezing-curve by experimental result, it can be recognized that both values are much agreed with each other.

Conclusion

It is concluded that to determine the time required for freezing of fishes regarded as a conical or a double conical body which is pasted together the same two conicals on their bases in shape like skip-jack, formulas and values of some physical and geometrical constants induced here are quite effective to calculate.